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Clinical Experience,

HER 2 in Gastric Cancer in Albania as a New Therapeutic Alternative

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Abstract:
Background: Gastric cancer (GC) is the fourth most common cancer in Albania. Gastric cancer has a5-year survival rate as low as 30%. The expression of HER2 in gastric cancer has brought a new alternative treatment for patients.
Materials and method: 192 patients were analyzed retrospectively, with primary GCs for HER2 overexpression by IHC and Dual SISH in equivocal cases. This was compared with the results of HER2 in gastric patients in surgical specimens and endoscopic biopsies and there is a correlationbetween gender, age, stage, and type of the histopathologic gastric cancer diagnosis.
Results: Examinations were made by immunohistochemistry for HER2 in 73.4% (141 cases) of surgical specimens and 26.5% of endoscopic biopsies: 18.4% (26 cases) and 15.7% (8 cases) were HER2 3+, respectively. HER2 overexpression (3+) was detected in 17.7% (34 cases). HER2 Equivocal (2+) was detected in 24.5% (47 cases). 17.8%, 14%, and 4.7% were respectively intestinal type, diffuse, signet ring, and the rest adenocarcinoma NOS. GC prevailed in the group aged 61-70 yrs. (31.70%), followed by 51-60 yrs. (25%), 22.9% in 71-80-yrs. 20 cases analyzed by SISH, showed HER2 amplification in 40% (8cases). Economical restrictions and problems with the preanalytical phase made it impossible to evaluate by SISH all 20 cases.
Conclusion: 17.7% of Albanian patients with primary GC were HER2-positive on IHC. There is no difference in biopsy and surgical specimen results. Economic restrictions can influence the results.

Keywords: immunohistochemistry, HER2, IHC, Gastric carcinoma, SISH

Abbreviations

HER2 - Human Epidermal growth factor receptor 2; IHC – Immunohistochemistry; SISH - Silver DNA in Situ Hybridization; DNA - Deoxyribonucleic acid; NOS - no special type; GC - Gastric cancer; Sc – Scoring; LDM -Logical Data Model; SHAP - Shapley Additive exPlanations is a visualization tool that can be used for making a machine learning model more explainable by visualizing its output; FISH - fluorescence in situ hybridization; NCCN - National Comprehensive Cancer Network

Introduction:

General Data: Stomach Adenocarcinoma is one of the most frequent neoplasms in the world. Its incidence varies greatly from country to country, with high incidence in Japan, Chile and Finland and low incidence in Thailand and in many parts of Africa. In the United States, there has been a steady decline in incidence of gastric cancer over the last 50 years, and currently it has 10 cases per 100000 people. [1] In Albania, in 2008, the population was estimated at 3,170,048. 45% of the population is urban, 55% is rural population according to INSTAT. The average life expectancy is 72.1 years for males and 78.6 years for women (2005-2008). (Ref) Gastric Cancer has a 5-year survival of 5-20%. There is not yet a

standard chemotherapy used all over the world for gastric cancer patients.

In patients without metastases and with tumors that can be resected surgically, the main therapy (with treatment purpose) has been surgery, followed by chemotherapy or radiotherapy, depending on the stage and tumor type. In recent years, the role of systemic treatment is stabilized and in many cases as primary treatment is used neoadjuvant chemotherapy followed by surgery. The antrum, body and fundus carcinoma can be divided into two histologic-intestinal and diffuse types (Lauren Classification – the causes of which are different, also have different precursor lesions and various growth speeds) [4]. The used terminology and histopathological assessment are subjective mainly for two important elements of carcinogenesis, atrophy and dysplasia. Microscopically, both in early gastric cancer and in advanced cancer, histological findings are similar, with their glandular form, from well-differentiated on average, and mis-differentiated, and a different subtype with cells "like ring with stones." [3] There are still no specific molecular markers for the diagnosis of gastric cancer, although the latter is rarely a challenge for the pathologist. HER2 / NEU amplification seems to be an indication of the ability for metastasis and poor prognosis. This may result in an effective target for molecular-based therapies [2].

Material and Methodology:

An epidemiological study on HER2 status in gastric cancer was conducted in Albania. This was a retrospective and prospective study, which consisted of collecting 192 cases (paraffin blocks) of endoscopic biopsy materials, but also operating parts of two central pathological-anatomy laboratories (LDM and SHAP, UHC "Mother Teresa"). All samples were examined for the expression of HER2 with immunohistochemistry and a portion of HER2/SISH. Anatomopathological reports were analysed to see the correlation between the presence of HER2 expression and clinicopathological traits. Immunohistochemical examination is performed by the manual method with the Hercep test ™ (Dako) anti-corpus, *(*HercepTest™ *pharmDx kits are available for immunohistochemical determination of HER2 protein overexpression using Dako Omnis),* Automated Link Platforms, Scoring Score (0) - one or little staining in < 10% of cells; Score (1+) - faint, partial staining in > 10% of cells; Score (2+) - weak to moderate, complete staining in > 10% of cells; Score (3+) - strong, complete membrane staining in > 10% of cells.) [5, 6, 7]

Grading systems differ depending on the type of cancer. In general, tumors are graded as 1, 2, 3, or 4, depending on the amount of abnormality. In Grade 1 tumors, the tumor cells and the organization of the tumor tissue appear close to normal. These tumors tend to grow and spread slowly. In contrast, the cells and tissue of Grade 3 and Grade 4 tumors do not look like normal cells and tissue. Grade 3 and Grade 4 tumors tend to grow rapidly and spread faster than tumors with a lower grade.If a grading system for a tumor type is not specified, the following system is generally used: GX: Grade cannot be assessed (undetermined grade); G1: Well differentiated (low grade); G2: Moderately differentiated (intermediate grade); G3: Poorly differentiated (high grade); G4: Undifferentiated (high grade) [8] TNM categories were determined according to the Japanese classification of gastric carcinoma which is widely used. In this classification, the depth of tumor invasion is recorded as the T category, lymph node metastasis is recorded as the N category, and the presence/absence and sites of distant metastasis are recoded as the M category. [9] All data is analysed with Microsoft Excel.

Results:

From 192 examined cases, the demographic data showed an average age of 58.4 years with predominance of the age group 60-71 (Table 1).

Table 1- Distribution of HER2 data in according the Age

Age (years)	*No. of cases*	*% of cases*
< 30	2	1.04
31-40	6	3.12
41-50	29	15.1
51-60	55	28.6
61-70	61	31.8
71-80	37	19.3
>81	2	1.04
Total	192	100

Of all examined cases; 69 cases (35%) are from endoscopic biopsy and 123 cases (65%) are operating parts. 9 cases (4.7%) are T4, 91 cases (47.4%) are T3, 23 cases (11.98%) are T2 and 69 cases (35%) biopsy without stratification (corresponding to endoscopic biopsy). Of all examined cases with IHC for HER2, there were HER2 in Score 0 with 53 cases, HER2 in Score 1+ with 57 cases, HER2 in Score 2+ with 47 cases and HER2 Score 3+ with 34 cases. (Table 2).

Table 2 - Distribution of HER2 data in according the score and SISH value.

HER2	*No. of cases*	*% of cases*
Score 0	53	27.6
Score 1+	57	29.7
Score 2+	47	24.5
Score 3+	34	17.7
NOS	1	0.2
SISH Positive	8	17
SISH Negative	12	25.5
SISH unspecified	27	57.5
SISH Total	47	100

Out of 47 cases HER2 in T2+ (equivoque) there were 20 of them where it could not be determined with IHC if there was amplification or not of the HER2 gene; after SISH examination the results were: SIS positive in 8 cases (17%) and Negative in 12 cases (15.5%). (Table 2)

From the correlation of the histological subtype with HER2 expression, it resulted that in the bad-differentiated G3 adenocarcinomas, there was overexpression of HER2 (HER2 3+), more than in those of G1 (respectively, 23.5% vs. 11.7%). Mucosal adenocarcinomas are mainly without amplification of the HER2 gene respectively, 15.2% HER2 Score 0 and 3.5% HER2 Score 1+, andzero cases with HER2 Score 3+.

Similar data is available for "ring with stone-like" cell carcinoma, where 2.9% of them are HER2 Score 3+ and 8.4% HER2 Score 0. Also, although adenocarcinoma of the intestinal subtype has a better prognosis than that of diffuse subtype, in our cases, 8.8% of intestinal ones present amplification of HER2 gene and 20.4% of diffuse infiltrative carcinomas do not have amplification ofHER2 gene (Table 3).

Table 3-Distribution of histological features data in according to stage of Gastric Cancer

HISTOLOGICAL FEATURES	T...N...M..		T4N...M..		T3N...M..		T2N...M..		TOTAL	
	No.	%	No.	%	No.	%	No.	%	No.	%
G1: Well differentiated;	10	14.5	-	-	5	5.49	1	4.35	16	8.34
G2: Moderately differentiated	23	33.3	1	11.1	25	27.47	7	30.42	56	29.2
G3: Poorly differentiated	12	17.4	-	-	35	38.46	7	30.42	52	27.1
Signet Ring Cell	7	10.15	3	33.3	1	1.1	1	4.35	12	6.25
Ca Mucinosis	2	2.9	1	11.1	13	14.28	2	8.7	18	9.5
Ca Mixed	-	-	-	-	2	2.2	1	4.35	3	1.56
Ca Diffuse	7	10.15	1	11.1	6	6.6	2	8.7	16	8.34
Ca Intestinalis	6	8.7	3	33.3	2	2.2	1	4.35	12	6.25
Ca unspecified	2	2.9	-	-	2	2.2	1	4.35	5	2.61
Total	69	35.94	9	4.7	91	47.4	23	11.98	192	100
	endoscopic biopsy		Operator biopsy							

In correlating the expression of HER2 with the anatomopathological stage, there is no significant difference between the stage and the expression of HER2 (HER2 Score 3+) in our cases (T4 2.9%, T335.3%, T2 8.8% and 52.5% unspecified). (Table 4, 5).

Table 4- Distribution of data in according to biopsy

HER2	*Surgical Specimen*		*Endoscopic Biopsy*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Score 0	40	28.36	10	19.6	50	26.1
Score 1+	47	33.33	14	27.45	61	31.7
Score 2+	35	24.82	12	23.52	47	24.5
Score 3+	19	13.47	15	29.41	34	17.7
Total	141	73.43	51	26.7	192	100

Table 5- Distribution of HER2 SISH data in according to biopsy

HER2 SISH	*Surgical Specimen*		*Endoscopic Biopsy*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Positive	3	8.57	5	41.66	8	17.02
Negative	8	22.86	4	33.33	12	25.53
Unspecified	24	68.57	3	35.0	27	57.44
Total	35	74.47	12	25.53	47	100

Discussion:

In our study the most affected age group with GC was 61-70 years old with 61 (31.8%) of cases. In according of one study of *Vishi et al.* the most affected age group is 51-70 years which includes about 61.6% of patients. [10] In patients with gastric cancer and gastroesophageal cancer, HER2 amplification identifies those patients who benefit from Trastuzumab therapy. HER2 status assessment is however influenced by pre-analytic and post-analytic parameters, as reported for breast cancer.[2]

In a study by *Kapelessor et.al.* Of the 5,426 tissue fragments examined by microprobes, HER2 was found to be more pronounced in intestinal type and low-grade cancers and had no correlation with age, sex, stage, and tumor localization. Even in a study of *Moelans CB and bp.*, a low frequency of HER2 expression was seen in early gastric cancer. In our cases the data was different, with amplification in the mis-differentiated subtypes and in the intestinal subtype. The above data can be explained by the biological characteristics of gastric cancer in Albania; another hypothesis is also the non-standardized TNM reporting protocol provides variable data that can influence the outcome. However, 17.7% of our patients have HER2 expressions and may be a group that can benefit from targeted therapy. [11]

HER2 is a key element (key drive of tumorigenesis and overexpression as a result of amplification of the Her-2 gene has been seen in a number of patients with solid tumors).[12]

HER2 is overexpressed in 7-34% of gastric cancer patients according to the literature [13, 14, 15], in our patients in 17.7% of cases. In many cases gastric cancer is diagnosed in locally advanced or metastatic stages which makes it even more important to perform the HER2 test. Actually, standard anti-HER2 therapy with -Trastuzumab combined with chemotherapy in advanced and metastatic gastric cancer is standard therapy. Results from randomized phase III studies, indicate a benefit and higher survival with Trastuzumab plus chemotherapy in cases of locally advanced, recurrent or metastatic gastric or gastro-oesophageal cancer with HER-2 overexpression. Based on this data, all patients diagnosed with gastric cancer should be tested for HER-2 status at the time of initial diagnosis [16, 17, 18]

We still do not test all cases since the moment of initial diagnosis, mainly from the lack of reagents to perform this examination. Another problem encountered is sometimes the inaccuracy in the staging, as a consequence of the imaging that sometimes results in the under-staging of cases and inability to receive [data] from the beginning the targeted therapy with Trastuzumab.

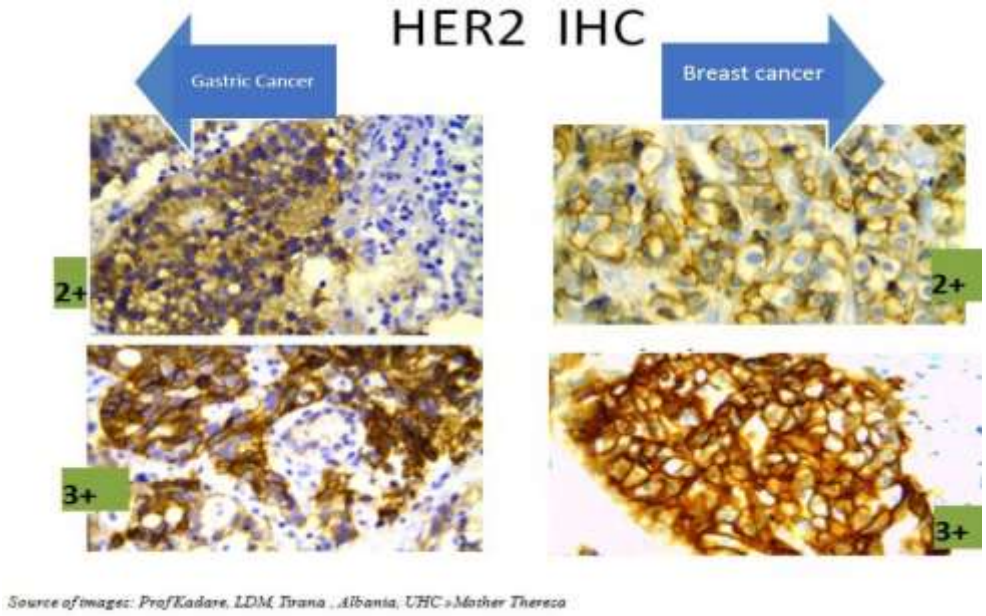

Figure 1-Difference in HER2 expression in gastric and breast cancer.

In order for the Her-2 test results to be accurate, strict rules should be followed according to the criteria of gastric cancer; this will also affect the setting of the treatment protocol and consequently may affect the survival of patients. Applying the same criteria for breast cancer can give false negative results (underscoring tumor) and may make it impossible to receive therapy with Trastuzumab. HER2 assessment is done with microscopic examination with a semiquantitative method by observing the intensity of the colour in the nuclear membrane, as well as the staining of theentire perimeter of this membrane.

There is a difference in the evaluation of HER2 in the materials from breast and gastric cancer. This difference is also reflected in the guidelines published for this examination. [19]

In endoscopic biopsies, which are small biopsies In a study by *Rüschoff J et al.,* variability is observed between the immunohistochemical methodand that with SISH. [20]

In a study by *Mrklic I. et al.,* for the evaluation of HER2 in gastric cancer and its scoring system, for the standardization of methodology, the results of the IHC method were compared with that of SISH, not only within the laboratory, but also between different centres that participated in this study. [21]

Conclusion:

HER2 testing has been performed for almost a decade in Albania, mainly for breast cancer. It has been switched from a manual to an automated with artifacts from oppression, especially in the periphery of the tissue, with inflammatory infiltrates, HER2 results may present artifacts that influence the outcome. Even in the guidelines, there is a difference in interpretation between small endoscopic biopsies and operative parts. In our material there is no significant difference in the expression of HER2 (HER2 Score 3+) between endoscopic biopsies and operative parts (respectively 15 and 19 cases, figure 2). In gastric cancer, HER2 status is partially influenced by variation in the used methodology, the instruments, and the experience of the laboratories conducting the testing. [20]

One of the purposes of conducting our study in two laboratories was quality control by comparing the results between laboratories and the methodologies used by each.

methodology. For many years it has served only a laboratory as a central immunohistochemistry laboratory. New centres are already being developed. HER2 is expressed in approximately 30% of breast cancer patients and 17.7% in those with gastric cancer. Sharing methodology between laboratories will lead to improved quality assurance in HER2testing.

COI Statement:

This paper has not been submitted in parallel. It has not been presented fully or partially at a meeting or podium or congress. It has not been published nor submitted for

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