THAI NGUYEN UNIVERSITY
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TRAN NGOC THUY

STUDY OF THE EXPRESSION OF C-MET, HER2, PCNA MARKERS AND THEIR RELATION WITH THE CLINICAL ENDOSCOPIC HISTOPATHOLOGICAL CHARACTERISTICS IN GASTRIC ADENOCARCINOMA PATIENTS

Speciality: Internal Medicine
Code: 9720107

ABSTRACT OF MEDICAL PHD THESIS

THAI NGUYEN - 2024

The thesis was done in:
Thai Nguyen University of Medicine and Pharmacy

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The thesis is defended in front of the Thesis Judging Committee at Thai Nguyen University, which meets at: Thai Nguyen University of Medicine and Pharmacy

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INTRODUCTION

Stomach cancer is a common cancer with a high mortality rate worldwide. Although there have been many advances in diagnosis and treatment, the prognosis for advanced gastric cancer remains poor with an average 5-year survival rate of about 10%.

HER2, an epithelial growth factor receptor belonging to the tyrosine kinase receptor class, has been shown to be overexpressed associated with poor prognosis in various cancers including gastric cancer. Likewise, C-MET also belongs to the receptor family of tyrosine kinases (RTK), which are growth factor receptors involved in many physiological responses essential for embryonic development and homeostasis,encoded by the MET gene. In gastric cancer, C-MET is considered as a potential target second only to HER2 in targeted therapy. Additionally, PCNA is known as a protein that plays an important role in cell division and growth. High PCNA expression is associated with clinical features and prognosis in gastric cancer patients.

The expression of C-MET, HER2 and PCNA are determined by immunohistochemistry in gastric cancer, as well as the association with clinical features endoscopic histopathology and cancer stage is still limitited, especially in Vietnam. Therefore, we conduct the project with the following objectives:

1. *Study the expression of markers C-MET, HER2, PCNA in patients with gastric adenocarcinoma stained by immunohistochemistry.*
2. *Compare the expression of markers C-Met, HER2, PCNA with clinical, endoscopic, histopathological findings in patients with gastric adenocarcinoma*

NEW CONTRIBUTION OF THE THESIS

- This is the first study on the simultaneous expression of C-MET HER2 and PCNA conducted in Vietnam.

- The results of the study showed that: The percentages expression of C-MET, HER2 and PCNA were: 51.3%, 28.7%; and 54.7% respectovely. The high expression rate of all three markers was 16.7%; 2/3 of the markers showed a rate of 28.7%, while only 1 marker showed an expression rate of 27.3%.

- Expression of C-MET, HER2, PCNA is related to histology according to Lauren and WHO classification. HER2 expression is associated with the degree of differentiation. Co-expression of C-MET, HER2, PCNA was associated with Lauren's and WHO histopathological type but not with tumor differentiation and TNM stage.

THESIS STRUCTURE

The thesis consists of 126 pages (excluding references and appendices), divided into 4 chapters (Introduction 2 pages, Overview 36 pages, Object and research methods: 23 pages, Research results 30 pages, discussion 32 pages, conclusion 2 pages, proposals 1 page), The results section includes 46 tables, 3 charts and 23 figures, The references section contains 139 documents (15 Vietnamese, 124 English), with 3 appendices.

MAIN ABBREVIATIONS

1. HER2 Human Epidermal Growth Factor Receptor 2
2. PCNA Prolifarating cell nuclear antigen
3. TNM Tumor–Node–Metastasis

CHAPTER I
OVERVIEW

1. C-MET in gastric cancer

C-MET (or MET) belongs to the family of receptor tyrosine kinases RTKs, capable of binding to HGF and activating the HGF/C-MET signaling pathway, thereby regulating proliferation and migration of tumor cells

According to many studies, high C-MET expression is associated with the prognosis of the patient's condition. Overexpression of C-MET has been reported to be strongly correlated with increased tumor growth, metastatic status, poor prognosis radiotherapy resistance of cancer, as well as increased tumor viability and disease recurrence.

According to Betts G's study, C-MET overexpression was seen in 4% of the study cases and was associated with reduced survival ($P < 0.001$). In the study by Nakajima et al, overexpression of C-MET was correlated with depth of tumor invasion and lymph node metastasis. The survival time of C-MET-overexpressing gastric cancer patients is worse than that of non-overexpressing gastric cancer patients. The above study found that there is a statistically significant correlation between the expression of C-MET and the poor prognosis in gastric cancer patients, thereby thinking that C-MET is a toxic prognostic factor established in gastric cancer.

C-MET is now considered an important target in targeted therapy against cancer. Several molecules targeting C-MET have recently been covered in early-stage clinical trials. Most of them are minor kinase inhibitors, while some are biologic antagonists and monoclonal antibodies.

2. HER2 in gastric cancer

HER2 is encoded by the ERBB2 gene located on chromosome 17, which is a proto-oncogene. HER2 encodes a transmembrane tyrosine kinase receptor, homologous to EGFR that is important for the growth, differentiation, and survival of malignant and normal epithelial cells.

The prognostic role of HER2 in gastric cancer is still variable between different studies. Overall, although some of the small studies mentioned above have not demonstrated the prognostic properties of HER2, a large number of recent studies have shown that HER2 is a negative prognostic factor.

HER2 expression has been used in predicting treatment response and has yielded positive results. When HER2 is expressed at high levels in the tumor, it is often indicated for targeted therapy with the monoclonal antibody trastuzumab. Trastuzumab is a monoclonal antibody against HER2. There is no consensus on the mechanism of action of trastuzumab in cancer cells, but evidence suggests that in addition to preventing dimerization of HER2 with members of the HER family and stimulating endocytosis, it appears to induce immunity mediates and inhibits angiogenesis.

In Vietnam, Le Viet Nho (2014) analyzed the expression of EGFR and HER2 by immunohistochemistry and showed that about 21% of gastric cancer cases studied were Her2 positive. This result has certain implications for the selection of HER2-targeted therapy with trastuzumab.

3. PCNA in gastric cancer

Cell growth nuclear antigen (PCNA) is a protein found in all eukaryotic species. PCNA has an integral role for DNA replication and maintenance of genome integrity in developing cells.

A recent meta-analysis study demonstrated that high PCNA expression is associated with higher mortality, and it may be a useful prognostic biomarker in glioma and cancer Cervical. However, in the case of gastric cancer, the impact of PCNA on patient survival and clinical characteristics is still controversial.

This meta-analysis included 19 individual studies with 2,852 patients on the relationship between PCNA and prognosis, as well as with clinical indicators in breast cancer. The results indicate that high expression of PCNA predicts Os and DFS poorly in cancer patients. Increased PCNA is correlated with deeper tumor invasion, lymph node metastasis, and advanced TNM stage. These findings further verified the association between high PCNA expression and poor OS, consistent with the results of many previous studies.

Currently, a number of PCNA-targeted inhibitors have been studied in recent times, which opens up new therapeutic opportunities for targeted therapy in gastric cancer patients. There are two types of PCNA-targeted inhibitors including peptides and small molecules. Therefore, these novel treatments could be further investigated for PCNA-targeted treatment in gastric cancer patients with high PCNA expression.

CHAPTER II
SUBJECTS AND METHODS

2.1. Studying subjects

During the period from January 2018 to June 2022, we conducted research on 150 patients diagnosed with gastric adenocarcinoma at K Hospital.

2.1.1. Selection criteria

- The patient's histopathology results after endoscopic tumor biopsy were determined by Hematoxylin-Eosin (HE) staining to be gastric adenocarcinoma. The patient is indicated for surgery to remove a gastric tumor. The patient agreed and voluntarily participated in the study. The specimen is a postoperative tumor processed according to standard procedures and immunohistochemically stained to determine the markers C-MET, HER2 and PCNA.

2.1.2. Exclusion criteria

- Stomach cancer patients have received treatment (chemotherapy, radiotherapy). Patients with metastatic stomach cancer from other places. Patients with recurrent stomach cancer. Postoperative specimens did not meet the requirements when processed and when subjected to immunohistochemical staining.

2.2. Research time and location

- Study period: From January 2018 to June 2022. Study location: K Hospital (Quan Su Facility) and Inserm U1053 Laboratory, National Institute of Health and Medical Research, Bordeaux, French Republic.

2.3. Reseach methodology

- Cross-sectional descriptive research method.
- Steps to conduct research

Clinical examination and subclinical indications

Patients who come to the hospital for examination will be asked about disease, clinical examination, indicated for hematology, biochemistry, coagulation, and immunological tests. The patient is assigned to have a gastroscopy, when a stomach tumor is found, a biopsy will be performed during the endoscopy.

Gastroendoscopy

Insert the gel-coated endoscope through the mouth into the esophagus, into the stomach, inflate, and closely observe the gastric mucosal areas. When a lesion is found, a detailed assessment of the anatomical location and image of the lesion is performed. When a lesion is detected, the pump is washed, then carefully observed with NBI mode and near focus to evaluate. Biopsy do pathology if in doubt.

Tumor removal surgery and how to handle specimens

Stomach cancer specimens after surgery were transferred to the Pathology Department at K Hospital for dissection, fixed in 10% formalin solution, transferred and molded in paraffin to form candle blocks for tissue testing pathology.

A tissue sample molded in paraffin from the patient was selected to be sent to the laboratory Inserm U1053 Institute for Health and Medical Research, Bordeaux, France. Conduct test histopathology and immunohistochemistry for C-met, HER2 and PCNA.

Expression analysis of C-MET, HER2, PCNA by immunohistochemistry

- Conducting immunohistochemical studies at Laboratory U1053 - National Institute of Health Sciences and Medical Research - Bordeaux France.

a. Paraffin removal

- Tissue slices were washed 3 times with xylene solution, 5 minutes each time
- Wash twice with 100% ethanol, 10 minutes each time
- Wash twice with 95% ethanol, 10 minutes each time
- Wash twice with water, 5 minutes each time

Note: Always avoid drying the tissue at any time during this process.

b. Antigen reveal

- Place slides containing paraffinized tissue slices in a container containing Citrate buffer pH6. These cans are placed in the pressure cooker and securely closed. Turn on pressure mode 950C – 980C for 30 minutes. Next, place the buffer solution box containing the slides outside in the environment for 30 minutes to allow the temperature to gradually decrease.
- Wash with TBST1X buffer, once in 5 minutes.
- Add enough (2-4 drops, equivalent to about 50 µL) of Hydrogen Peroxide Block solution to cover the surface of tissue slices. Wash twice with 1X TBST buffer, 5 min each time.
- Add 50 µl Protein blockK and incubate for 10 min at room temperature to prevent nonspecific staining. Wash once with 1X TBST buffer.
- Add 50 µl of antibody solution 1 anti …… mixed in Protein Block solution, incubate for 1 h at room temperature. Wash twice with TBST buffer, 5 min each time.
- Add 50 µl of Biotinylated Goat Anti-Mouse solution and incubate for 10 minutes at room temperature. Next wash with 1X TBST buffer (twice, 5 min each).

- Add 50 µl of Streptavidin Peroxidase and incubate for 10 minutes at room temperature, then wash 4 times with TBST buffer, 5 minutes each time.

- Add 1 tbsp of DAB Chromogen solution to 1.5 ml of DAB substrate, vortex evenly and aspirate 50 µl of the solution after mixing to cover the cut tissue. Incubate for 5 minutes at room temperature. Wash 4 times with 1X TBST buffer, 5 min each time.

- Place slides containing tissue slices in Hematocylin solution for 3 minutes.

- Dewatering

- Incubate slides containing tissue slices in 95% ethanol solution, repeat 2 times, 3 minutes each time.

- Incubate in 100% ethanol solution, repeat 2 times, 3 minutes each time.

- Incubate the xylene solution 2 times, 3 minutes each time.

-Mount the slide with SignalStain Mounting Medium solution.

Interpretation of immunohistochemical staining results under optical microscope at 100-400x magnification was performed by an experienced pathologist.

2.4. Research indicators and variables

- Tumor morphology according to Borrmann:

+ Borrmann type I (Polyp form), Borrmann type II (Fungal form), Borrmann type III (Ulcerative form),Borrmann type IV (Infiltrative form).

- Histopathological classification according to Lauren:

+ Intestinal type, Diffuse type. Indeterminate type.

- Histopathological classification according to the WHO 2019 classification system:

+ Papillary adenocarcinoma.

+ Tubular adenocarcinoma.

+ Mucinous adenocarcinoma.

+ Signet-ring cell adenocarcinoma.

+ Mixed carcinoma

- Histopathological classification according to the degree of differentiation according to WHO: Poor differentiated. Moderately differentiated. Well differentiated.

- Diagnosis of TNM stage: According to the 8th AJCC system.

- Assess the expression level of marker HER2 in cancer samples to levels: 0, 1+, 2+, 3+. The expression level of HER2 is 0 and 1+ evaluates to negative, the expression level of HER2 is 2+ and 3+ evaluates to positive.

- Assess the expression level of marker C-MET and PCNA in cancer samples to levels: 0, 1+, 2+, 3+. The expression level of HER2 is 0 and 1+ evaluates to low expression, the expression level of HER2 is 2+ and 3+ evaluates to high expression.

2.5. Research ethics

The research topic has been approved and approved by the Ethics Council and Scientific Council of the University of Medicine and Pharmacy - Thai Nguyen University.

2.6. Data analysis

The data were processed using the SPSS 20.0 medical statistical software.

CHAPTER III
RESEARCH RESULT

3.1. Clinical, endoscopic and histological features of patients with gastric adenocarcinoma in the antrum

- The rate of gastric cancer in men is more common than in women and the age group is 60-69. The incidence of the disease in rural areas is almost twice higher than in urban areas.

- Most patients have a history of epigastric pain and/or persistent dyspepsia.

- The main reason for hospital admission is most often epigastric pain, accounting for 70%.

- The number of patients with the time from the first symptom to hospital admission <3 months accounted for the highest rate (58%).

- Epigastric pain, weight loss, anorexia, are common symptoms, with the rate of 99,3%, 48%, 38%, respectively. Other symptoms are less common.

- According to the Borrmann classification, the majority of lesions had ulcerative appearance (53,3%), followed by fungal form (36%), infiltrative form (8%) and the lowest was polypoid form (2,7%)).

- According to Lauren's histopathological classification, intestinal type accounted for a higher proportion than diffuse type (72,7% versus 22,7%).

- Small tubular adenocarcinoma accounts for the highest percentage (58,7%), followed by other types.

- Low-differentiated gastric cancer accounts for the highest proportion (51,6%), followed by moderately differentiated (42,9%) and the lowest is highly differentiated (5,5%).

- Cancer: T2 to T4 (100%), lymph node metastasis from N1 to N3 (66%) and some distant metastases from M1 (2%). Stage II to stage IV (98,7%).

3.2. Expression of C-MET, HER2, and PCNA in gastric cancer

Table 3.11. Co-expression of C-MET, HER2 and PCNA in gastric cancer

Simultaneous expression marker	**Marker**	**n=150**	**Rate %**	**n=150**	**Rate %**
	0	41	27,3	41	27,3
C-MET	1	41	27,3	19	12,6
HER2				4	2,7
PCNA				18	12
C-MET, HER2	2	43	28,7	4	2,7
C-MET, PCNA				29	19,3
HER2, PCNA				10	6,7
C-MET, HER2, PCNA	3	25	16,7	25	16,7

The high expression rate of all 3 markers is 16,7%; 2/3 of the marker is 28,7%; 1 marker (27,3%).

3.3. Compare the expression of markers C-MET, HER2, PCNA with clinical characteristics, endoscopic images, and histopathology

Table 3.26. Compare C-MET expression with histopathological characteristics according to the Lauren classification

C-MET / Lauren	Low expression		High expression		n = 150		p
	n	%	n	%	n	%	
Intestinal type	47	43,1	62	56,9	109	100	0.001
Diffuse type	25	73,5	9	26,5	34	100	
Indeterminate type	1	14,3	6	85,7	7	100	

Indeterminate type has a higher rate of C-MET expression (85,7%) than that of intestinal type (56,9%) and diffuse type (26,5%). The difference was statistically significant with $p < 0,05$.

Table 3.27. Compare C-MET expression with histopathological characteristics according to WHO classification

C-MET / WHO	Low expression		High expression		n = 150		p
	n	%	n	%	n	%	
Papillary	1	33,3	2	66,7	3	100	0,004
Tubular	40	45,5	48	54,5	88	100	
Mucinous	6	33,3	12	66,7	18	100	
Signet-ring cell	25	73,5	9	26,5	34	100	
Mixed	1	14,3	6	85,7	7	100	

Mixed carcinoma had the highest C-MET expression rate at 85.7%. papillary and mucinous at 66,7%, tubular 54,5% and ring cells 26,5%. The difference was statistically significant ($p < 0,05$).

Table 3.29. Compare HER2 expression with histopathological characteristics according to Lauren classification

HER2 / Lauren	negative		positive		n = 150		p
	n	%	n	%	n	%	
Intestinal type	73	67	36	33	109	100	0,025
Diffuse type	30	88,2	4	11,8	34	100	
Indeterminate type	4	57,1	3	42,9	7	100	

Indeterminate type has a higher rate of HER2 expression (42,9%) than that of intestinal type (33%) and diffuse type (11,8%). The difference was statistically significant with $p < 0,05$

Table 3.30. Compare HER2 expression with histopathological characteristics according to WHO classification

HER2 / WHO	negative		positive		n = 150		p
	n	%	n	%	n	%	
Papillary	1	33,3	2	66,7	3	100	0,040
Tubular	61	69,3	27	30,7	88	100	
Mucinous	11	61,1	7	38,9	18	100	
Signet-ring cell	30	88,2	4	11,8	34	100	
Mixed	4	57,1	3	42,9	7	100	

Papillary adenocarcinoma has the highest HER2 expression rate (66,7%), mixed (42.9%), mucinous 38,9%, tubular form 30,7% and lowest is Signet ring cell 11.8%. The difference was statistically significant ($p < 0,05$).

Table 3.31. Compare HER2 expression with tumor differentiation

HER2 / Tumor differentiation	negative		positive		n = 91		p
	n	%	n	%	n	%	
Well	2	40,0	3	60,0	5	100	0,013*
Moderately	22	56,4	17	43,6	39	100	
Poor	38	80,9	9	19,1	47	100	

High expression of HER2 in highly differentiated tumors (60,0%), moderately differentiated (43,6%), low differentiated (19,1%) with $p < 0.05$

Table 3.32. Compare PCNA expression with histopathological characteristics according to Lauren classification

PCNA / Lauren	Low expression		High expression		n = 150		p
	n	%	n	%	n	%	
Intestinal type	42	38,5	67	61,5	109	100	0,002
Diffuse type	24	70,6	10	29,4	34	100	
Indeterminate type	2	28,6	5	71,4	7	100	

Indeterminate type has a higher rate of PCNA expression (71,4%) than that of intestinal type (61,5%) and diffuse type (29,4%). The difference was statistically significant with $p < 0,05$

Table 3.33. Compare PCNA expression with histopathological characteristics according to WHO classification

PCNA / WHO	Low expression		High expression		n = 150		p
	n	%	n	%	n	%	
Papillary	0	0	3	100	3	100	0,002
Tubular	32	36,4	56	63,6	88	100	
Mucinous	10	55,6	8	44,4	18	100	
Signet-ring cell	24	70,6	10	29,4	34	100	
Mixed	2	28,6	5	71,4	7	100	

Papillary adenocarcinoma has the highest PCNA expression rate (100%), mixed and tubular (71,4% and 63,6%), Mucinous 44,4% and signet ring cell is 29,4%. The difference was statistically significant ($p < 0,05$).

Table 3.35. Compare co-expression of C-MET, HER2, PCNA with histopathological characteristics according to Lauren classification

Lauren	Number of positive marker				p
	0 marker	1 marker	2 marker	3 marker	
Intestinal (n=109)	23 (21,1%)	29 (26,6%)	35 (32,1%)	22 (20,2%)	0,001
Diffuse (n=34)	17 (50%)	12 (35,3%)	4 (11,8%)	1 (2,9%)	
Indeterminate (n=7)	1 (14,3%)	0 (0%)	4 (57,1%)	2 (28,6%)	

The simultaneous co-expression of all three markers was highest in the indetermoinate type (28,6%) than in the intestinal

type (20,2%) and diffuse type (2,9%). The difference was statistically significant (p < 0,05).

Table 3.36. Compare co-expression of C-MET, HER2, PCNA with histopathological characteristics according to WHO classification

WHO	Number of positive marker				p
	0 marker	**1 marker**	**2 marker**	**3 marker**	
Papillary (n=3)	0 (0%)	0 (0%)	2 (66,7%)	1 (33,3%)	0,002
Tubular (n=88)	20 (22,7%)	22 (25%)	29 (33%)	17 (19,3%)	
Mucinous (n=18)	3 (16,7%)	7 (38,9%)	4 (22,2%)	4 (22,2%)	
Signet-ring cell (n=34)	17 (50%)	12 (35,3%)	4 (11,8%)	1 (2,9%)	
Mixed (n=7)	1 (14,3%)	0 (0%)	4 (57,1%)	2 (28,6%)	

There was a difference in the simultaneous expression of all 3 markers according to WHO histopathological characteristics, p < 0,05.

CHAPTER IV

DISCUSSION

4.1. Clinical, endoscopic and histological features of patients with gastric adenocarcinoma in the antrum

4.1.1. General characteristics of the study subjects

Gastric cancer is a gender-related gastrointestinal malignancy, with a higher predisposition to males than females. In this study, the male/female ratio was 1.94/1. This result is consistent with a number

of national and international studies, with the male/female ratio ranging from 1.8/1-3.0/1.0.

In our study, the average age of gastric cancer patients was 59.4 ± 11.7 years, with the majority of gastric cancer patients concentrated in the age group over 50 years old, accounting for 82%. In which, the rate of stomach cancer in the age group 60-69 years old accounted for the highest rate was 34,7%. Through the assessment of gender and age characteristics, we noted that the older age group, male sex is Important factors in the diagnosis of gastric cancer.

Our study found that the incidence of gastric cancer in rural areas is 66%, and in urban areas is 34%. This result is also consistent with other authors. Research by Nguyen Lam Hoa at Viet Tiep Hai Phong Hospital with the rate of gastric cancer in rural areas is 77,6%.

4.1.2. Clinical features

Most patients have a history of diseases or symptoms suggestive of an increased risk of gastric cancer. There were 10,7% of patients with a history of gastritis, 2% of gastric ulcers, 1 case had gastric surgery. According to Do Trong Quyet, the rate of patients with gastric cancer with a history of stomach ulcers accounts for 11,4%. Le Viet Nho percentage of patients with a history of gastric ulcer (10,0%), chronic gastritis (10,0%)

There are 4 main reasons to bring gastric cancer patients to hospital in order: epigastric pain (accounting for 70% of total patients), weight loss (accounting for 20.7% of total patients), gastrointestinal bleeding (6,7%) and difficulty swallowing (2.6%).

In this study, the number of patients with the time from the first symptom to hospital admission <3 months accounted for the highest percentage (58%). The results of our study are similar to those of

other authors. According to Le Viet Nho, the disease duration <3 months accounted for the highest rate (77,8%).

Patients with gastric cancer often have no specific clinical symptoms. The most common systemic and systemic symptoms in our study included epigastric pain, followed by weight loss, anorexia, nausea and/or vomiting. Less common symptoms include anemia and difficulty swallowing. The rate of common symptoms in our study is quite consistent with the research results of domestic authors, as well as some foreign authors.

4.1.3. Endoscopic imaging features

In this study, according to the Borrmann classification, Ulcerative form (53,3%), fungal form (36%), infiltrative form (8%) and Polyp form (2,7%). Our results are similar to many domestic studies. In the study of Nguyen Lam Hoa, the macroscopic image of the fungus accounted for the highest proportion (43.1%), followed by the ulcer form (40.6%).

4.1.4. Histopathological features

According to Lauren's histopathological classification, we noted that the intestinal type accounted for a higher proportion than the diffuse type (72,7% versus 22,7%).

In Vietnam, previous studies also often noted that intestinal type is more dominant than diffuse type. A recent study by Le Viet Nho also noted that intestinal type accounted for a higher rate than diffuse type (51,1% versus 48,9%). In the study of Nguyen Ngoc Hung (2007), intestinal type was significantly more than diffuse type (73% vs 27%) similar to our study. Studies in Vietnam have many differences in the rate of histology of gastric cancer according to

Lauren's classification. More studies with larger sample sizes are needed to get more accurate conclusions.

Based on WHO histopathological classification in 2010, we noted: tubular adenocarcinoma form is the most common (58,7%). The results of our study are similar to that of some domestic and foreign authors, with small tubular carcinoma accounting for the highest percentage. In Vietnam, Pham Minh Anh (2022) studied on 142 patients and determined that tubular adenocarcinoma for the majority (64.1%), mucinous adenocarcinoma (12,7%), Signet ring cells carcinoma (7,7%), papillaryadenocarcinoma (12%). Other bodies account for only a very low percentage. Nguyen Ngoc Hung (2007) histopathological survey on 300 gastric cancer samples also found that tubular carcinoma form accounts for the highest percentage (50,7%), followed by other types..

In our study, Poor differentiated (51,6%), Moderately differentiated (42,9%) andWell differentiated (5,5%). This result is similar to the study Lazăr and Lee K.E. Poor differentiation with rates of 63% and 47%.

4.2. Expression of C-MET, HER2, and PCNA in gastric cancer

In Fuse's study, there was a statistically significant difference between the HER2 (+) / C-MET (+) co-expressing group and the HER2 (+) / C- MET (-) co-expressing group (P = 0.043). And C-MET (+) is considered a poor prognostic factor. In another study of Ha, the rate of simultaneous expression of both C- MET, HER2 and EGFR was 15/169. Positive rates: HER2 (9%) and C- MET (22%) and EGFR (17%) . In this study, the combination of HER2 inhibitor (Lapatinib) + C- MET inhibitor (PHA665752) was better at inhibiting gastric tumor cells than lapatinib alone. In Jia's study, the

co-expression ratio of HER2/ C-MET was 7%. Furthermore, HER2(+) / C-MET (+), HER2(+)/ FGFR2(+) and triple-positive status were significantly associated with poor clinical outcomes when the cohort was subdivided by co-existence condition.

In this study, 72,7% of the patients (109/150) expressed at least one of the three markers and 16.7% of the patients (25/150) simultaneously had three highly expressed markers. Simultaneous expression of 2 out of 3 C-MET markers, HER2 and PCNA accounted for 28,7%, of the 2 co-expressed markers, C-MET and PCNA were both positive, accounting for 19.3%, highest among 2 markers with the same expression. There is no sign of expression, the rate is 27,7%. So far, there have been almost no studies on the co-expression of all 3 markers C-MET, HER2 and PCNA, so we do not have data to compare.

4.3. Compare the expression of markers C-MET, HER2, PCNA with clinical characteristics, endoscopic images, and histopathology

The results of our study show that in gastric cancer the expression of C-MET, HER2, PCNA is not related to sex. Among our gastric cancer patients, the expression rates of C-MET, HER2 did not differ between age groups. While PCNA expression was different between age groups ($p < 0.05$). Through studying domestic and international studies, although age and sex are two factors associated with the risk of stomach cancer, most studies suggest that neither age nor sex associated with the expression of markers in gastric cancer.

Considering the correlation between C-MET, HER2 and PCNA with clinical symptoms of the group of patients in the study, we found: although the expression of C-MET, HER2 and PCNA is

associated with cancer prognosis. But C-MET, HER2 and PCNA expression were not associated with clinical symptoms of gastric cancer patients.

Like many studies in the world today, we did not find a relationship between macroscopic features on endoscopy and the expression of C-MET, HER2 and PCNA.

Expression of C-MET, HER2, PCNA was associated with histology according to Lauren and WHO classification ($p < 0.05$). While HER2 expression, PCNA was associated with the degree of differentiation. This result is similar to many studies around the world. Kataoka's study recorded a higher rate of HER2 positivity in the intestinal type than in the diffuse type. And the rate of HER2 expression of small ductal carcinoma, papillary type (21.7% and 50%), while mucinous and undifferentiated form, there were no cases of HER2 expression ($p < 0.0001$). According to Ha (2013) study on 495 patients studied. Overexpression of C-MET in the study was associated with Lauren's histopathological classification ($p = 0.001$).

The co-expression of C-MET, HER2, PCNA was associated with histopathological type according to Lauren's and WHO classifications but not with tumor differentiation ($p > 0.05$). This suggests that C-MET, HER2 and PCNA are related to the development and progression of histopathological characteristics of cancerous tumors. Targeting a single target has some limitations in the treatment of gastric cancer due to the complex pathogenesis of the disease. Thus, single-targeted drugs are more likely to lose their effectiveness immediately after the compensatory mechanism is activated. This is one of the main reasons leading to the failure of

single-targeting in gastric cancer. Therefore, we believe that with a deep understanding of the molecular mechanism of gastric cancer, there will be breakthroughs in the multi-targeted treatment of gastric cancer in the future, thereby opening a new phase in gastric cancer treatment.

Regarding the relationship between C-MET expression and cancer stage, we found: C-MET expression is not associated with stage N, stage M; C-MET expression in patients with stage T4 was higher than in patients with stage T2 and T3 ($p < 0.05$). In this study, HER2 and PCNA expression was not associated with T stage, N stage, M stage. HER2 and PCNA expression was also not associated with cancer stage ($p > 0.05$). This result is also consistent with many other studies.

CONCLUSION

1. Expression of markers C-MET, HER2, PCNA in gastric adenocarcinoma

+ The rate of high expression C-MET, HER2, PCNA respectively: 51,3%; 28,7%; and 54,7%. The high expression rate of all 3 markers is 16,7%; 2/3 of the marker is 28,7%; 1 marker (27,3%).

+ C-MET expression level 1+, 2+, 3+ has the rate: 16,7%; 27,3% and 24%.

+ HER2 expression levels 1+, 2+, 3+ have the rate: 14%; 19,3% and 9,3%.

+ PCNA expression levels 1+, 2+, 3+ have the rate: 40,7%; 18% and 36,7%.

2. Compare the expression of markers C-MET, HER2, PCNA with clinical characteristics, endoscopic images, and histopathology

+ Expression of C-MET, HER2 is not related to sex, age. PCNA expression was different between the age groups in the study ($p < 0,05$).

+ The expression of C-MET, HER2, PCNA was not associated with the gross tumor characteristics according to the Borrmann classification.

+ Expression of C-MET, HER2, PCNA is associated with histology according to Lauren classification and WHO.

+ HER2 expression is related to the degree of differentiation.

+ C-MET expression was associated with T stage but C-MET, HER2, PCNA expression was not associated with TNM stage of gastric cancer ($p > 0,05$).

+ The co-expression of C-MET, HER2, PCNA was associated with histopathological type according to Lauren and WHO classification but not with tumor differentiation and TNM stage ($p > 0,05$).

PROPOSALS

Through this study, we have some recommendations as follows:

In addition to HER2 assessment, which has been used frequently in clinical practice, C-MET and PCNA markers should be added to collect useful information for diagnosis, prognosis and influence on targeted treatment.

Expand research and evaluate the prognostic value of the markers C-MET, HER2 and PCNA in early gastric cancer.

LIST OF PUBLISHED ARTICLE RELATING TO THESIS

1. Tran Ngoc Thuy, Nguyen Phu Hung, Duong Hong Thai (2022), "Clinical, endoscopic and histological features of patients with gastric adenocarcinoma in the antrum". *TNU Journal of Science and Technology*, 228(1), pp.18-23.

2. Tran Ngoc Thuy, Nguyen Phu Hung, Duong Hong Thai (2022), "C-MET expression in gastric adenocarcinoma". *Vietnam Medical Journal*, 521(1), pp.69-73.

3. Tran Ngoc Thuy, Nguyen Phu Hung, Le Phong Thu, Duong Hong Thai (2022), "PCNA expression in gastric adenocarcinoma". *Vietnam Medical Journal*, 521(1), pp.214-217.

4. Tran Ngoc Thuy, Nguyen Phu Hung, Duong Hong Thai (2024), "HER2 expression in gastric adenocarcinoma". *TNU Journal of Science and Technology*, 229(1), pp.251-258.