ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

HER-2/neu expression in different histological subtypes of papillary thyroid carcinoma

DELIA CIOBANU APOSTOL[1,2)], IRINA-DRAGA CĂRUNTU[1)], LUDMILA LOZNEANU[1,2)], ELENA CORINA ANDRIESCU[1,2)], SIMONA ELIZA GIUŞCĂ[1)]

[1)]*Department of Morphofunctional Sciences I, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[2)]*Department of Pathology, "Sf. Spiridon" Emergency County Hospital, Iaşi, Romania*

Abstract

The identification and validation of new, complementary prognostic factors represents a challenging issue in thyroid pathology, opening the perspective of papillary thyroid carcinoma (PTC) stratification based on differences of aggressiveness at molecular level. Our study aims to analyze the HER-2/neu expression in different subtypes of PTC and its relationship with the classical clinicopathological factors. We investigated 120 cases of sporadic PTC. The cases were selected based on the histological criteria reported to clinical course and prognosis and distributed in two different subgroups, namely low- and high-risk. HER-2/neu expression was assessed using an adapted semiquantitative score proposed for thyroid carcinomas. The correlations between HER-2/neu expression and clinicopathological prognostic factors were statistically analyzed. HER-2/neu positivity was found in 25 (20.8%) cases, from which 20 cases were classified as subtypes with low-risk and five with high-risk; 95 (79.2%) cases were HER-2/neu negative, 53 cases being included in low- and 42 cases, respectively, in high-risk group. HER-2/neu expression was significantly associated with histological subtypes, extrathyroidal extension, and tumor focality. Our results highlight the potential of HER-2/neu as supplementary marker useful for the stratification of PTC in low-risk and high-risk histological groups, along with the well-known clinicopathological prognostic factors. Our data could be considered as new evidences that indicate different involvement of HER-2/neu in tumor development and behavior, possible due to its connection with other factors present in the molecular environment.

Keywords: papillary thyroid carcinoma, histological subtypes, HER-2/neu, prognostic factor.

Introduction

Thyroid carcinomas, originating from the follicular cells, are the most common endocrine tumors. They account for 1–2% of all malignancies [1–3], and more than 90% are differentiated forms, classified according to the histopathological criteria in two main groups: papillary thyroid carcinomas (PTCs) and follicular thyroid carcinomas (FTCs). PTC is the most frequent type, representing more than 70% of thyroid malignancies [3, 4]. The prognosis of PTC is good. However, some cases have an unexpected, aggressive evolution, with early lymph node and/or distant metastasis, and poor survival [5–7]. Therefore, the identification of new, complementary prognostic factors can be regarded as a challenging issue in thyroid pathology [8–11].

The differences in the PTC clinical progress, namely the low and high, respectively, metastasis potential, could be explained through particular events that appear in the carcinogenic mechanism and induce the alterations of the cellular cycle, changes in the cell adhesiveness, and involvement of the tumor microenvironment factors – yielding higher capacity of proliferation and dissemination of tumor cells. From the wide panel of molecular "candidate" prognostic factors, only two (namely cyclin D1 and p27, which intervene in the cellular cycle) are validated and already added to the classical clinical and morphological prognostic factors established by the *World Health Organization* (WHO) [3, 12], while the others are still under study [8–11]. This ongoing process is critical, because the validation of new prognostic factors opens the perspective of PTC stratification, based on differences of aggressiveness at molecular level.

Proto-oncogene HER-2/neu (C-erbB2), also known as CD340, is located on the 17q chromosome and it codifies the transmembrane tyrosine kinase receptor for the epidermal growth factor (EGF) [13]. Its amplification or overexpression plays an important part in tumor development, progression and aggressiveness, through direct effects on the cell cycle, angiogenesis, cellular motility and apoptosis [13].

HER-2/neu is considered a new prognostic factor in numerous types of cancer. It is involved in tumor biology, with a key role in the uncontrolled cell growth. HER-2/neu overexpression, firstly demonstrated in breast and ovary cancer [14–16] and later confirmed in gastric, colon, lung and bladder cancer [16–24] is associated with poorly differentiated phenotype, high metastasis capacity and poor overall survival [16]. Moreover, HER-2/neu becomes a therapeutic target in the breast and gastric cancer [16, 25, 26].

HER-2/neu overexpression in PTC has been identified in the aggressive forms with an increased potential of metastasis, allowing the supplementary Herceptin therapy for these patients, similar to breast cancer [16]. However, data on HER-2/neu involvement in PTC are controversial. HER-2/neu overexpression is reported in a wide range of values, varying from 0% to 79% [27–33]. These discre-

pancies are due to the large differences in the assessment methodology [27–30, 32–39]. Consequently, there is no consensus on the prognostic and therapeutic value of HER-2/neu [33, 39, 40].

Within this context, our study aims to analyze the HER-2/neu expression in different subtypes of PTC and its relationship with the classical clinicopathological factors.

Patients, Materials and Methods

Patients

The study group included 120 selected cases of sporadic papillary thyroid carcinoma, diagnosed at the "Sf. Spiridon" Emergency County Hospital, Iaşi, Romania, between 2010 and 2016. All patients were surgically treated; from the entire study group 52 patients underwent total thyroidectomy and 68 patients total thyroidectomy with regional lymphadenectomy. The selection process was based on the histological criteria, following to include subtypes with indolent course and good prognosis and, respectively, with aggressive behavior and poor prognosis. The cases were distributed in two different subgroups, namely low- and high-risk, by relying on the characteristics of the histological subtypes reported to clinical course and prognosis [9, 10, 41].

All cases were reviewed by three independent pathologists in order to establish the histological variant and to reassess the following characteristics: multifocality, lympho-vascular invasion, extrathyroidal extension (presence of tumor cells into perithyroidal soft tissues). Other clinicopathological data (gender, age, tumor size, and lymph node metastasis) were extracted from medical files. The cases were classified in accordance with the WHO Guidelines, the *Classification of Malignant Tumors* (TNM), and the *College of American Pathologists* Clinical Guidelines [3, 42]. The study has been approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, based on patients' informed consent.

Immunohistochemistry

For each case, a representative paraffin-embedded tissue fragment was chosen. The 3-μm thick sections obtained from the blocks were placed on silanized slides, dewaxed in xylene, rehydrated in consecutive descending concentrations of ethanol (100%, 90%, 80%, and 70%), and rinsed in distillated water. For antigen retrieval, we used Heat Induced Epitope Retrieval (HIER) technique: slides were placed in citrate buffer pH 6 and heated in a water bath, at 98^0C, for 30 minutes. The slides were immersed in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase, and incubated with the primary antibody for HER-2/neu (rabbit monoclonal, clone SP3, Thermo Scientific, USA), at 1:100 dilution, overnight, at 4^0C. The immunoreaction was amplified with the suitable secondary and tertiary antibodies of the UltraVision Quanto Detection System HRP DAB (Thermo Scientific, USA) and developed with 3.3'-diaminobenzidine (DAB) tetrahydrochloride chromogen (Thermo Scientific, USA). The counterstaining of the sections was done with Lillie's modified Hematoxylin. Positive and negative controls have been simultaneously run in order to verify the accuracy of the technique.

Semi-quantitative assessment

The evaluation of HER-2/neu expression was based on an adapted semiquantitative score proposed for thyroid carcinomas [29, 38], that assess not only membranous, but also cytoplasmic immunopositivity. This score was based on the percent of positive tumor cells (P), classified as 0 for <10%, 1 for >10% and <25%, 2 for >25% and <50%, 3 for >50% and <75% and 4 for >75% positive cells, respectively, and the staining intensity (I), categorized as 1 – weak, 2 – moderate, and 3 – strong. The final score was obtained as a product between P and I, with the following values: 0 – negative, 1–4 – weakly positive, 5–8 – moderately positive, 9–12 – strongly positive. These values were extrapolated to the scoring system usually applied for HER-2/neu in breast and gastric cancer [43]. Consequently, taking into account both cytoplasmic and/or membranous HER-2/neu staining, we considered the values between 0 and 8 as negative (corresponding to HER-2 1+ and HER-2 2+) and those between 9 and 12 as positive (corresponding to HER-2 3+).

Statistical analyses

Data were analyzed by univariate analysis, using the *chi*-square test (Maximum-Likelihood, Yates, Mantel–Haenszel) (SPSS V.19, SPSS Inc., IBM Corporation, Chicago, IL, USA). Statistical significance was considered for $p<0.05$.

Results

Clinicopathological characteristics of the study group

In the study group, the female gender was predominant – 93 (77.5%) cases, as compared to male one – 27 (22.5%) cases. The mean age at the time of the diagnosis was 53.11±14.87 years. Tumor size ranged between 8 and 100 mm, with a mean of 27.06±20.34 mm. Multifocality was present in 50 (41.66%) cases, extrathyroidal extension in 82 (68.33%) cases, and approximately a half of the investigated cases had lympho-vascular invasion [62 (51.66%) cases]. From the 68 patients who underwent total thyroidectomy with regional lymphadenectomy, lymph node metastases were found in 32 (47.05%) patients – N1a in 21 patients and N1b in 11 patients. Accordingly to pT stage, the cases were distributed as follows: pT1 – 22 (18.33%) cases, pT2 – eight (6.66%) cases, pT3 – 82 (68.33%) cases and pT4 – eight (6.66%) cases.

Among the patients included in our study, we identified 10 histological variants of PTC: conventional – 16 (13.33%) cases, follicular – eight (6.66%) cases, oncocytic – 25 (20.83%) cases, microcarcinoma – 21 (17.5%) cases, clear cell – three (2.5%) cases, tall cell – 15 (12.5%) cases, columnar cell – three (2.5%) cases, cribriform – six (5%) cases, hobnail – one case (0.83%), diffuse sclerosing – five (4.16%) cases, solid – six (5%) cases, angioinvasive follicular – seven (5.83%) cases, conventional with dedifferentiation to squamous cell carcinoma – three (2.5%) cases, oncocytic with undifferentiated solid areas – one case (0.83%).

The low-risk group (conventional, follicular, oncocytic, microcarcinoma, clear cell), with indolent behavior and good prognostic, included 73 (60.83%) cases, and the high-risk group (tall cell, columnar cell, cribriform, diffuse sclerosing, solid, hobnail, angioinvasive follicular, conventional with dedifferentiation to squamous cell carcinoma, oncocytic with undifferentiated solid areas) with aggressive behavior and poor outcome, 47 (39.17%) cases.

HER-2/neu expression

HER-2/neu expression assessed with score values between 1 and 4, considered negative, was heterogeneous, weak, with cytoplasmic location and granular pattern, visible in the apical and lateral domains of the tumor follicular cells (Figure 1).

In cases scored between 5 and 8, also considered negative, HER-2/neu expression was also heterogeneous, but moderate as staining intensity, with granular and diffuse cytoplasmic and discontinuous membranous patterns (Figure 2).

Cases scored between 9 and 12, considered positive, showed strong immunostaining of tumor cells, with both cytoplasmic and continuous, complete membranous distribution (Figure 3).

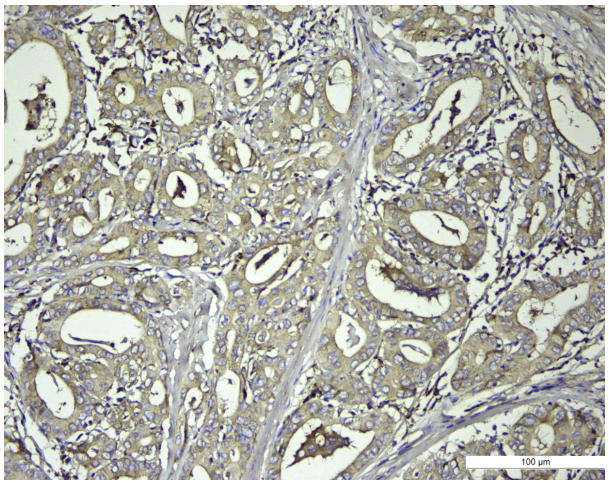

Figure 1 – ***Tumor cells with weak cytoplasmic granular pattern of HER-2/neu. IHC staining, anti-HER2, ×200.***

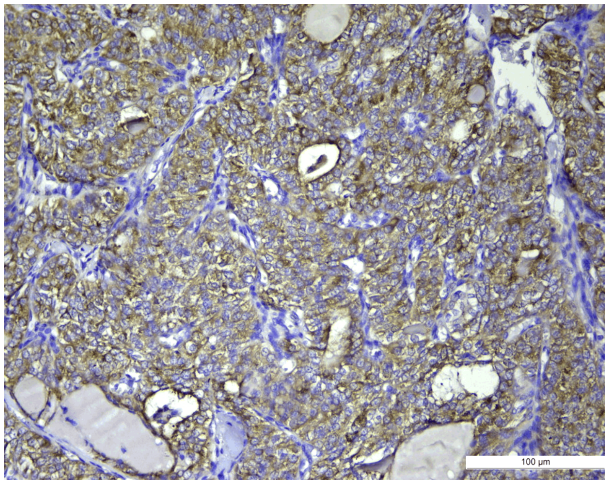

Figure 2 – ***Moderate HER-2/neu staining in the tumor cells, with predominant cytoplasmic pattern and focal, incomplete membranous immunoreactivity. IHC staining, anti-HER2, ×200.***

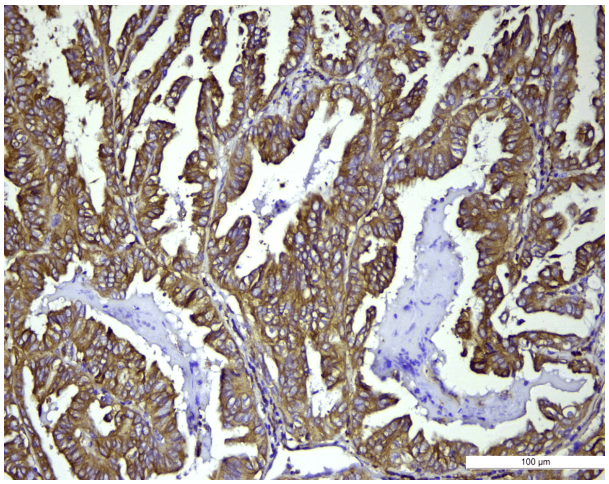

Figure 3 – ***Strong HER-2/neu expression: membranous pattern, with evident, complete membrane staining in tumor cells. IHC staining, anti-HER2, ×200.***

HER-2/neu positivity was noticed in 25 (20.8%) cases, from which 20 cases were classified as subtypes with low-risk and five with high-risk (Table 1). HER-2/neu was negative in 95 (79.2%) cases, distributed as follows: 53 cases as low-, and 42 cases, respectively, as high-risk subtypes (Table 1).

Table 1 – ***HER-2/neu expression in different subtypes of papillary thyroid carcinoma (PTC)***

Histological subtypes	HER-2/neu expression	
	Positive (n=25)	***Negative (n=95)***
Low risk group	*20*	*53*
▪ conventional (*n*=15)	5	11
▪ follicular (*n*=8)	3	5
▪ oncocytic (*n*=25)	6	19
▪ papillary microcarcinoma (*n*=21)	6	15
▪ clear cell (*n*=3)	0	3
High risk group	*5*	*42*
▪ tall cell (*n*=15)	1	14
▪ columnar cell (*n*=3)	0	3
▪ cribriform morular (*n*=6)	0	6
▪ diffuse sclerosing (*n*=5)	0	5
▪ solid (*n*=6)	1	5
▪ PTC with prominent hobnail features (*n*=1)	0	1
▪ angioinvasive follicular (*n*=7)	1	6
▪ conventional with dedifferentiation to squamous cell carcinoma (*n*=3)	2	1
▪ oncocytic with undifferentiated solid areas (*n*=1)	0	1

Correlations between HER-2/neu expression and clinicopathological prognostic factors

Univariate analysis revealed significant differences between HER-2/neu expression (positive *versus* negative) and histological subtypes, extrathyroidal extension, and tumor focality (Table 2). No significant correlation was found between HER-2/neu expression and age, gender, tumor size, lympho-vascular invasion, tumor stage, as well as lymph node metastases (Table 2).

Table 2 – ***Relationship between HER-2/neu expression and clinicopathological characteristics***

Clinicopathological characteristics	HER-2/neu expression		*Chi*-square test
	Negative (n=95)	***Positive (n=25)***	
Age at diagnosis			
▪ <55 years old	51 (85%)	9 (15%)	*p*=0.088
▪ ≥55 years old	44 (73.33%)	16 (26.66%)	
Gender			
▪ males	18 (66.7%)	9 (33.33%)	*p*=0.064
▪ females	77 (82.8%)	16 (17.2%)	
Tumor size (median)			
▪ <22 mm	49 (79.03%)	13 (20.97%)	*p*=0.575
▪ ≥22 mm	46 (79.31%)	12 (20.69%)	
Histological subtype			
▪ low-risk group	26 (61.9%)	16 (38.1%)	*p*=0.001*
▪ high-risk group	69 (88.5%)	9 (11.5%)	
Lympho-vascular invasion			
▪ absent	45 (77.59%)	13 (22.41%)	*p*=0.425
▪ present	50 (80.65%)	12 (19.35%)	
Extrathyroidal extension			
▪ absent	35 (92.1%)	3 (7.9%)	*p*=0.013*
▪ present	60 (73.17%)	22 (26.83%)	
Focality of the tumor			
▪ unifocal	61 (87.14%)	9 (12.86%)	*p*=0.011*
▪ multifocal	34 (68%)	16 (32%)	
Tumor stage			
▪ T1 + T2	26 (86.67%)	4 (13.33%)	*p*=0.183
▪ T3 + T4	69 (76.67%)	21 (23.33%)	
Lymph node metastases			
▪ N0	25 (69.44%)	11 (30.56%)	*p*=0.201
▪ N1	26 (81.25%)	6 (18.75%)	

*p-value <0.05 was considered to be statistically significant.

Discussion

HER-2/neu as an essential marker in the molecular classification of breast cancer [44] is overexpressed in 15–30% of invasive forms [45] and its role of prognostic and predictive factor is already accepted. Extensive researches demonstrated that HER-2/neu overexpression correlates with the disease stage, number of metastatic axillary lymph nodes, histological type, absence of estrogen and progesterone receptors and recurrence risk [16]. Consequently, the value of HER-2/neu as a therapeutic target reformed the breast cancer treatment, improving the clinical outcome [46, 47].

Starting with the '90s, several studies analyzed the HER-2/neu expression in ovary [15], gastric, colonic and esophageal [17–21], endometrial [48], lung [22, 23], and bladder [24] tumors. These studies sustain that HER-2/neu overexpression is associated with a more aggressive disease, incomplete response to primary therapy and worse overall survival – particularly in ovarian cancer [49].

The mechanism underlying the specific role of HER-2/neu in the thyroid carcinogenesis is still unknown. A large heterogeneity in HER-2/neu expression, varying between 0% and 79%, is reported [27–30, 32, 33, 35, 39, 40, 43].

The most studied histological type is the PTC, followed by FTC, medullary carcinoma, and anaplastic carcinoma, with contradictory and confusing results. The following three examples are relevant in support of this remark. Ruggeri *et al.* show a significantly higher HER-2/neu expression rate in the FTC compared to PTC [39], whereas Utrilla *et al.* [35] and Mdah *et al.* [33] report HER-2/neu positivity in 52%, respectively 6.9% of the analyzed PTC cases, but no expression in FTC. These differences are coming from the great variability of the size and general characteristics of the studied groups, and from the discrepancies in the methods used for the HER-2/neu scoring, including the subjectivity of evaluation [27–30, 32–39]. There is no agreement for HER-2/neu scoring in thyroid [40]. An important aspect is the cytoplasmic staining, frequently considered as positive immunoreaction in the HER-2/neu assessment [28, 29, 34, 35, 38]. However, the cytoplasmic immunoreactivity of HER-2/neu, possible due to the presence of a distinct protein in the mitochondrial cristae [50], is not yet explained. Therefore, other studies use the updated ASCO–CAP (*American Society of Clinical Oncology*/*College of American Pathologists*) scoring system applied in breast cancer [39, 43].

The controversial results lead to the lack of consensus regarding the HER-2/neu involvement, as prognostic and predictive factor and also as therapeutic target, in thyroid carcinoma in general and PTC in particular [33, 39, 40]. However, recent data show a relationship between HER-2/neu and BRAFV600E mutation in familial PTC with aggressive behavior [51], and sustain the HER-2/neu overexpression even in the absence of the gene amplification [40].

The present work focused on the HER-2/neu expression in different histological subtypes of PTC and its relationship with classical clinicopathological prognostic factors. In our series, we obtained HER-2/neu positivity in 20.8%, similar to the results reported in other previous papers – namely 18% [39], 19.7% [40], and 23% [32].

To the best of our knowledge, there are no data about the particular aspects of HER-2/neu expression in rare variants of PTC associated with an aggressive course, except for the tall cell subtype [40]. Therefore, it is worth to mention that in our study HER-2/neu overexpression was found mainly in non-aggressive histological subtypes of PTC (20 of the total number of 25 cases) in comparison with the aggressive ones. This observation complements the present data on HER-2/neu expression in PTC, and sustains the association between HER-2/neu and histological subtypes with good prognosis.

The role of HER-2/neu as prognostic factor falls in the same framework of controversies. Several studies report no HER-2/neu association with age, gender, tumor size [33, 39], tumor stage [29–32, 40], lymph node metastasis

[30–33, 39, 40], histological type [29–33], extrathyroidal extension [30, 32], and patient survival [40]. However, there are some positive results that sustain the association between the HER-2/neu in PTC and FTC and the distant metastasis [29], extrathyroidal invasion and tumor recurrences [39, 52].

Most of our results are similar with the negative findings of the above-mentioned studies. We found no relationship between HER-2/neu expression and age, gender, tumor size, lympho-vascular invasion, tumor stage, and lymph node metastases. On the other hand, we demonstrate that HER-2/neu overexpression correlates with the histological subtypes with a better clinical course, with extrathyroidal extension, and tumor focality.

Thus, our results highlight the potential of HER-2/neu as supplementary marker useful for the stratification of PTC in low-risk and high-risk histological groups, along with the well-known clinicopathological prognostic factors. Our data could be considered as new evidences that indicate different involvement of HER-2/neu in tumor development and behavior, possible due to its connection with other factors present in the molecular environment [51]. Certainly, we are aware that further, larger researches are compulsory to validate HER-2/neu as independent prognostic or predictive factor in PTC. Even if this process can be difficult, it is motivated by the practical consequences in diagnosis and therapy.

Conclusions

Our study sustains the HER-2/neu large heterogeneity in PTC. The correlation between HER-2/neu expression and different histological subtypes, extrathyroidal extension, and tumor focality supports its potential prognostic and predictive value, with promising application in the PTC stratification.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgments

This work was funded by "Grigore T. Popa" University of Medicine and Pharmacy under grant number 31584/2015.

References

[1] Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, Bai Y, Zhu C, Guo GL, Rothman N, Zhang Y. International patterns and trends in thyroid cancer incidence, 1973–2002. Cancer Causes Control, 2009, 20(5):525–531.

[2] Sherman SI. Thyroid carcinoma. Lancet, 2003, 361(9356): 501–511.

[3] De Lellis RA, Williams E. Thyroid and parathyroid tumours. In: De LellisRA, Lloyd RV, Heitz PU, Eng C (eds). Pathology and genetics of tumours of endocrine organs. World Health Organization (WHO) Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, 2004, 50–66.

[4] Romero-Rojas A, Cuervo-Martínez J, Osorio-Arango K, Olaya N. Histological variants and prognostic factors of papillary thyroid carcinoma at the Colombian Instituto Nacional de Cancerología, 2006–2012. Biomedica, 2015, 35(3):429–436.

[5] LiVolsi VA. Papillary thyroid carcinoma: an update. Mod Pathol, 2011, 24(Suppl 2):S1–S9.

[6] Lloyd RV, Buehler D, Khanafshar E. Papillary thyroid carcinoma variants. Head Neck Pathol, 2011, 5(1):51–56.

[7] Roman S, Sosa JA. Aggressive variants of papillary thyroid cancer. Curr Opin Oncol, 2013, 25(1):33–38.

[8] Sethi K, Sarkar S, Das S, Mohanty B, Mandal M. Biomarkers for the diagnosis of thyroid cancer. J Exp Ther Oncol, 2010, 8(4):341–352.

[9] Nikiforov YE. Molecular analysis of thyroid tumors. Mod Pathol, 2011, 24(Suppl 2):S34–S43.

[10] Radu TG, Mogoantă L, Busuioc CJ, Stănescu C, Grosu F. Histological and immunohistochemical aspects of papillary thyroid cancer. Rom J Morphol Embryol, 2015, 56(2 Suppl): 789–795.

[11] Radu TG, Ciurea ME, Mogoantă SŞ, Busuioc CJ, Grosu F, Ţenovici M, Petrescu IO, Vladu IM. Papillary thyroid cancer stroma – histological and immunohistochemical study. Rom J Morphol Embryol, 2016, 57(2 Suppl):801–809.

[12] Dean DS, Hay ID. Prognostic indicators in differentiated thyroid carcinoma. Cancer Control, 2000, 7(3):229–239.

[13] Yarden Y. The EGFR family and its ligands in human cancer. Signaling mechanisms and therapeutic opportunities. Eur J Cancer, 2001, 37(Suppl 4):S3–S8.

[14] Yokota J, Yamamoto T, Toyoshima K, Terada M, Sugimura T, Battifora H, Cline MJ. Amplification of c-erbB-2 oncogene in human adenocarcinomas *in vivo*. Lancet, 1986, 1(8484):765–767.

[15] Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, Clarke-Pearson DR, Marks P, McKenzie S, Yin S, Bast RC Jr. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res, 1990, 50(13):4087–4091.

[16] Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. Mol Biol Int, 2014, 2014:852748.

[17] Reichelt U, Duesedau P, Tsourlakis MCh, Quaas A, Link BC, Schurr PG, Kaifi JT, Gros SJ, Yekebas EF, Marx A, Simon R, Izbicki JR, Sauter G. Frequent homogenous HER-2 amplification in primary and metastatic adenocarcinomas of the esophagus. Mod Pathol, 2007, 20(1):120–129.

[18] Cervera P, Fléjou JF. Changing pathology with changing drugs: tumors of the gastrointestinal tract. Pathobiology, 2011, 78(2):76–89.

[19] Ieni A, Barresi V, Caltabiano R, Caleo A, Bonetti LR, Lanzafame S, Zeppa P, Caruso RA, Tuccari G. Discordance rate of HER2 status in primary gastric carcinomas and synchronous lymph node metastases: a multicenter retrospective analysis. Int J Mol Sci, 2014, 15(12):22331–22341.

[20] Ieni A, Barresi V, Giuffrè G, Caruso RA, Lanzafame S, Villari L, Salomone E, Roz E, Cabibi D, Franco V, Certo G, Labate A, Nagar C, Magliolo E, Broggi B, Fazzari C, Italia F, Tuccari G. HER2 status in advanced gastric carcinoma: a retrospective multicentric analysis from Sicily. Oncol Lett, 2013, 6(6):1591–1594.

[21] Ieni A, Barresi V, Rigoli L, Caruso RA, Tuccari G. HER2 status in premalignant, early, and advanced neoplastic lesions of the stomach. Dis Markers, 2015, 2015:234851.

[22] Heinmöller P, Gross C, Beyser K, Schmidtgen C, Maass G, Pedrocchi M, Rüschoff J. HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II study of herceptin. Clin Cancer Res, 2003, 9(14): 5238–5244.

[23] Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, Stevens C, O'Meara S, Smith R, Parker A, Barthorpe A, Blow M, Brackenbury L, Butler A, Clarke O, Cole J, Dicks E, Dike A, Drozd A, Edwards K, Forbes S, Foster R, Gray K, Greenman C, Halliday K, Hills K, Kosmidou V, Lugg R, Menzies A, Perry J, Petty R, Raine K, Ratford L, Shepherd R, Small A, Stephens Y, Tofts C, Varian J, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Knowles M, Leung SY, Louis DN, Looijenga LH, Malkowicz B, Pierotti MA, Teh B, Chenevix-Trench G, Weber BL, Yuen ST, Harris G, Goldstraw P, Nicholson AG, Futreal PA, Wooster R, Stratton MR. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature, 2004, 431(7008):525–526.

[24] Coogan CL, Estrada CR, Kapur S, Bloom KJ. HER-2/neu protein overexpression and gene amplification in human transitional cell carcinoma of the bladder. Urology, 2004, 63(4):786–790.

[25] May FE. Novel drugs that target the estrogen-related receptor alpha: their therapeutic potential in breast cancer. Cancer Manag Res, 2014, 6:225–252.

[26] Bilici A. Treatment options in patients with metastatic gastric cancer: current status and future perspectives. World J Gastroenterol, 2014, 20(14):3905–3915.
[27] Haugen DR, Akslen LA, Varhaug JE, Lillehaug JR. Expression of c-erbB-2 protein in papillary thyroid carcinomas. Br J Cancer, 1992, 65(6):832–837.
[28] Lemoine NR, Wyllie FS, Lillehaug JR, Staddon SL, Hughes CM, Aasland R, Shaw J, Varhaug JE, Brown CL, Gullick WJ, Wynford-Thomas D. Absence of abnormalities of the c-*erbB*-1 and c-*erbB*-2 proto-oncogenes in human thyroid neoplasia. Eur J Cancer, 1990, 26(7):777–779.
[29] Kremser R, Obrist P, Spizzo G, Erler H, Kendler D, Kemmler G, Mikuz G, Ensinger C. Her2/neu overexpression in differentiated thyroid carcinomas predicts metastatic disease. Virchows Arch, 2003, 442(4):322–328.
[30] Mondi MM, Rich R, Ituarte P, Wong M, Bergman S, Clark OH, Perrier ND. HER2 expression in thyroid tumors. Am Surg, 2003, 69(12):1100–1103.
[31] Balta AZ, Filiz AI, Kurt Y, Sucullu I, Yucel E, Akin ML. Prognostic value of oncoprotein expressions in thyroid papillary carcinoma. Med Oncol, 2012, 29(2):734–741.
[32] Sugishita Y, Kammori M, Yamada O, Poon SS, Kobayashi M, Onoda N, Yamazaki K, Fukumori T, Yoshikawa K, Onose H, Ishii S, Yamada E, Yamada T. Amplification of the human epidermal growth factor receptor 2 gene in differentiated thyroid cancer correlates with telomere shortening. Int J Oncol, 2013, 42(5):1589–1596.
[33] Mdah W, Mzalbat R, Gilbey P, Stein M, Sharabi A, Zidan J. Lack of HER-2 gene amplification and association with pathological and clinical characteristics of differentiated thyroid cancer. Mol Clin Oncol, 2014, 2(6):1107–1110.
[34] Sugg SL, Ezzat S, Zheng L, Rosen IB, Freeman JL, Asa SL. Cytoplasmic staining of erbB-2 but not mRNA levels correlates with differentiation in human thyroid neoplasia. Clin Endocrinol (Oxf), 1998, 49(5):629–637.
[35] Utrilla JC, Martín-Lacave I, San Martín MV, Fernández-Santos JM, Galera-Davidson H. Expression of c-erbB-2 oncoprotein in human thyroid tumours. Histopathology, 1999, 34(1):60–65.
[36] Elliott DD, Sherman SI, Busaidy NL, Williams MD, Santarpia L, Clayman GL, El-Naggar AK. Growth factor receptors expression in anaplastic thyroid carcinoma: potential markers for therapeutic stratification. Hum Pathol, 2008, 39(1):15–20.
[37] Qin C, Cau W, Zhang Y, Mghanga FP, Lan X, Gao Z, An R. Correlation of clinicopathological features and expression of molecular markers with prognosis after ^{131}I treatment of differentiated thyroid carcinoma. Clin Nucl Med, 2012, 37(3): e40–e46.
[38] Wu G, Wang J, Zhou Z, Li T, Tang F. Combined staining for immunohistochemical markers in the diagnosis of papillary thyroid carcinoma: improvement in the sensitivity or specificity? J Int Med Res, 2013, 41(4):975–983.
[39] Ruggeri RM, Campennì A, Giuffrè G, Giovanella L, Siracusa M, Simone A, Branca G, Scarfì R, Trimarchi F, Ieni A, Tuccari G. HER2 analysis in sporadic thyroid cancer of follicular cell origin. Int J Mol Sci, 2016, 17(12):2040.
[40] Siraj AK, Beg S, Jehan Z, Prabhakaran S, Al-Sobhi SS, Al-Dawish M, Al-Nuaim A, Al-Dayel F, Sauter G, Al-Kuraya KS. The role of *HER2* overexpression in Middle Eastern papillary thyroid cancer. Transl Cancer Res, 2017, 6(2):366–373.
[41] Kakudo K, Tang W, Ito Y, Mori I, Nakamura Y, Miyauchi A. Papillary carcinoma of the thyroid in Japan: subclassification of common type and identification of low risk group. J Clin Pathol, 2004, 57(10):1041–1046.
[42] Brierley JD, Gospodarowicz MK, Wittekind C (eds). TNM classification of malignant tumours. 8th edition, Wiley–Blackwell, 2017.
[43] Rakha EA, Starczynski J, Lee AH, Ellis IO. The updated ASCO/CAP guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. Histopathology, 2014, 64(5):609–615.
[44] Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature, 2000, 406(6797):747–752.
[45] Burstein HJ. The distinctive nature of HER2-positive breast cancers. N Engl J Med, 2005, 353(16):1652–1654.
[46] O'Sullivan CC, Swain SM. Pertuzumab: evolving therapeutic strategies in the management of HER2-overexpressing breast cancer. Expert Opin Biol Ther, 2013, 13(5):779–790.
[47] Kim M, Agarwal S, Tripathy D. Updates on the treatment of human epidermal growth factor receptor type 2-positive breast cancer. Curr Opin Obstet Gynecol, 2014, 26(1):27–33.
[48] Buza N, Roque DM, Santin AD. HER2/neu in endometrial cancer: a promising therapeutic target with diagnostic challenges. Arch Pathol Lab Med, 2014, 138(3):343–350.
[49] English DP, Roque DM, Santin AD. HER2 expression beyond breast cancer: therapeutic implications for gynecologic malignancies. Mol Diagn Ther, 2013, 17(2):85–99.
[50] De Potter CR, Van Daele S, Van de Vijver MJ, Pauwels C, Maertens G, De Boever J, Vandekerckhove D, Roels H. The expression of the *neu* oncogene product in breast lesions and in normal fetal and adult human tissues. Histopathology, 1989, 15(4):351–362.
[51] Caria P, Cantara S, Frau DV, Pacini F, Vanni R, Dettori T. Genetic heterogeneity of HER2 amplification and telomere shortening in papillary thyroid carcinoma. Int J Mol Sci, 2016, 17(10):1759.
[52] Akslen LA, Myking AO, Salvesen H, Varhaug JE. Prognostic impact of EGF-receptor in papillary thyroid carcinoma. Br J Cancer, 1993, 68(4):808–812.

Corresponding author
Irina-Draga Căruntu, Professor, MD, PhD, Department of Morphofunctional Sciences – Histology, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Phone +40727–003 700, e-mail: irinadragacaruntu@gmail.com

Received: December 15, 2016

Accepted: July 9, 2017