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MOLECULAR GENETIC CHARACTERISTICS OF GASTRIC CANCERS FROM THE SURGEON'S POINT OF VIEW

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Summary

A study on the expression of oncoproteins in stomach` carcinomas, has been turned into a real research basically due to the abundance of results and their comprehensive interpretations. The study carried out on the abdominal onco-surgical department of Odessa Regional Oncology Center, included a study of 188 patients operated on for gastric cancer (GC) between 2007-2011. In all cases was performed the so-called lymphadenectomy for the principal reason of extensive preventive biopsy of visually unchanged lymph nodes. We spend a multivariate analysis of interactions between the expression of oncoproteins p53, VEGFR-3, erbB2, Ki67 and micro involvement of tumor vasculature (ly, v), the local growth (T), the presence of residual tumor tissue (the R), the degree of tumor differentiation (the G) the degree of regional lymph nodes involvement (N) and type of infiltration (Inf α, β, Inf Y).

Keywords: Stomach cancer, Immunohistochemistry and Oncoproteins.

Gastric cancer (GC) genetical characteristic study shows largely speculative, theorized character, remaining "armchair science". It's clinical significance is revealed mostly in predicting chemotherapy or screening for therapeutic and preventive measures respectively.

Significant expression her2 \ new - trastuzumab, lapatinib administration; EGRF mutated gene – prescribing of Erlotinib, Gefitinib, Afatinib; translocation of EML4-ALK - Krizotinib; translocation of BCR-ABL - Imatinib; "Wild" type tyrosine kinase gene K-RAS and N-RAS -

predictors of efficacy of Cetuximab and Panitumumab , a serine-threonine kinase B-RAFwt - Vemurafenib; the presence of gene mutations CDH-1, BRCA-1, MLH1 , MLH3, MSH-2, MSH-6, PMS-1 - inherited gastric cancer predisposition, etc. In the processing - novel theraputical "targets": COX-2 inhibitors (there are reports on the effectiveness of Aspirin when mutated PIK3CA), metalloproteinases , telomerase, farnesyltransferase.

COX-2 (COX-2) along with VEGFR-3 (VEGF-C) is considered to be a prognostic factor in gastric cancers. There are a number of reports on the prognostic role of HER-2 marker in gastric cancers [26, 27, 28, 29].

ERCC1 expression is referred to as a predictor of sensitivity to Oxaliplatin [32] in stomach cancer.

The level of mRNA BRCA-1 gene in gastric cancer in clinical trials had a negative correlation with respect to the sensitivity of GC to Cisplatin and positive - with respect to sensitivity to Taxanes. There is a case described in the medical literature of successful cure BRCA-1 positive metastatic gastric cancer with multiple liver lesions by Docetaxel [44], see Fig. 1.

Jong Gwang Kim [18] described the following target molecules, expired in advanced gastric cancer (2013):

1. A monoclonal antibody to the receptor EGFR (Cetuximab / Panitumumab).
2. Tyrosine Kinase Inhibitor EGFR (Erlotinib / Gefitinib).
3. Monoclonal antibody to the receptor Anti-HER-2 mAbs (Trastuzumab).
4. tyrosine kinase inhibitors of HER-2 TKI (Lapatinib)
5. Monoclonal antibodies to growth factor Anti-VEGF (Bevacizumab).
6. The monoclonal receptor antibody Anti-VEGFR (Ramucirumab).
7. Inhibitors of tyrosine kinase receptor VEGFR (Sunitinib / Sorafenib / Cediranib / Apatinib).
8. Everolimus –mTOR inhibitor (mammalian target of Rapamycin), Akt pathway.
9. Onartuzumab - monoclonal antibody to hepatocyte growth factor receptor (HGFR), encoded by the c-Met oncogene.

And some other molecules, are still at the stage of clinical trials. Ongoing multicenter study KEYNOTE-012 (1b phase), dedicated to the prospects of Pembrolizumab immunotherapy in patients with advanced PD-L1 + GC [41].

Genetic testing for the individualization program and screening of healthy people should be done just once per life (full-genetic sequencing). On the contrary, tumor cell population is heterogeneous, there is a subpopulation of cells in various stages of cell cycle, with different sensitivity to chemotherapeutic regiments [7]. Differences between chemo-naive and pretreated cancers (after formation of drug resistance) motivated us to obtain repeated biopsies and promote

the gradual implementation of the immunohistochemical study of biopsies of tumors [5], including gastric cancer.

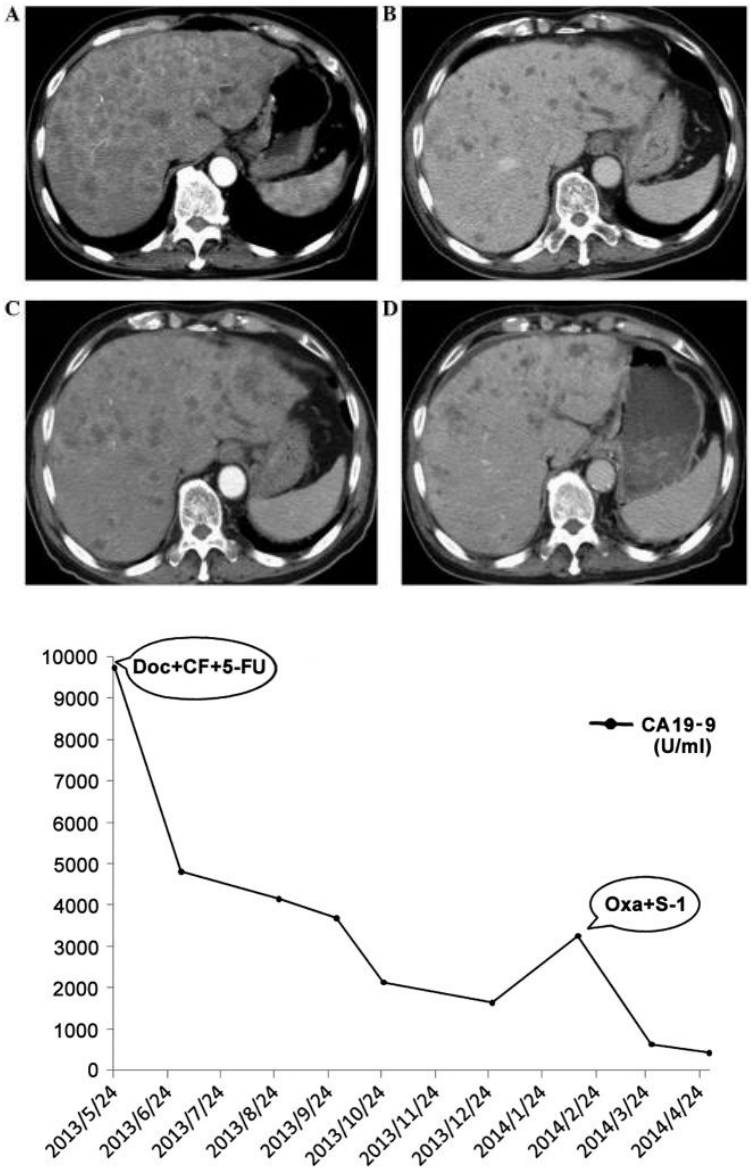

Figure 1. Complete clinical and instrumental regression in a patient with the BRCA-1 positive metastatic gastric cancer with liver disease on the background of Docetaxel therapy.

The introduction into clinical practice the molecular classification of gastric cancer [9], such as what is widely used in breast cancers, makes this study direction actualistic.

It was impossible in one study to consider all variants of protein molecules that are expressed in stomach cancer [10, 11]. Taking into account the new genetic classification, the four most well-known affordable oncoproteins were distinguished. Genetic classification of gastric cancer includes four types of tumors namely:

1. The genetically stable stomach cancer.

2. Microsatellite-unstable stomach cancer.

3. Chromosomal-unstable stomach cancer.

4. Epstein-Barr virus-associated gastric cancer.

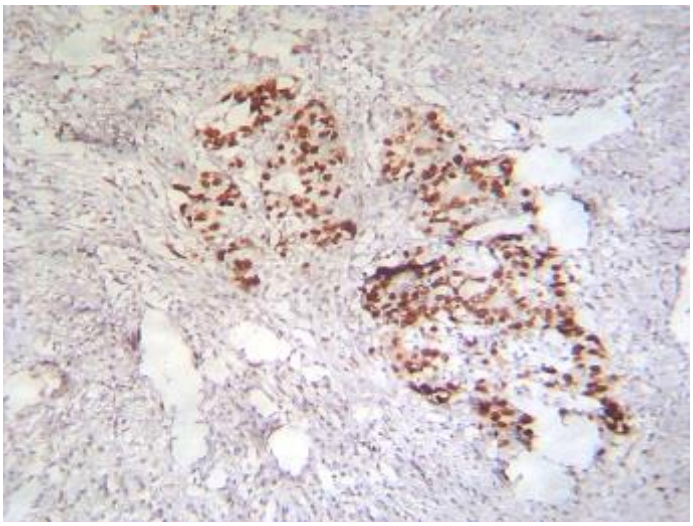

Fig. 2. Immunohistochemical staining for p53 Protein (DO-7) - oncoprotein expression level with a molecular weight of 53 kDa here 80%.

For example, chromosomally unstable gastric cancer (CUN) shows amplification of the TP53 gene (short arm of chromosome 17) and oncogene her2\new (long arm of chromosome 17) - respectively, for the study proteins p53 and erbB2 (CD 340) are eligible, see Figures №2 and №3. Chromosome unstable gastric cancer (CUN) takes approximately 50% of all malignant epithelial tumors of the stomach, so the study of these two markers appears to be very effective.

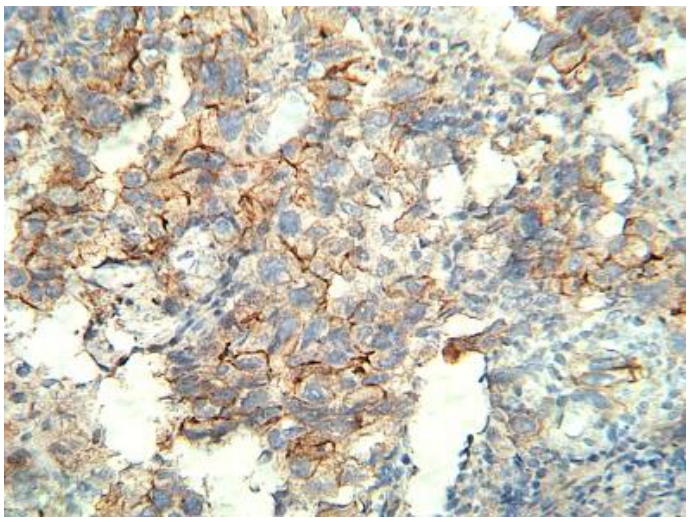

Fig. 3. Immunohistochemical staining for Her-2neu (with 2-erB-Oncoprotein) - 3+.

Three other genetic forms of gastric cancer: microsatellite unstable cancer (MSU) [16, 38, 43]; genetically stable cancer ("cancer of Napoleon" [36] - CDH-1 induced, the protein product is E-cadherin), or as it is called "cadherin" gastric cancer; and Epstein-Barr (EBV) virus-induced

gastric cancer [13, 14, 15, 43], wherein the gene expression is observed from the other receptor signaling cascade epidermal EGRF, - PI3K or PIK3CA, - phosphoinositol-3-kinase.

The most frequently observed mutations at microsatellite unstable gastric cancer is the so-called "silencing» of the main DNA repair genes - MLH1. Turn off other repair genes - MLH3, MSH2, MSH3, MSH6, PMS1, PMS2 (although, strictly speaking, all suppressor genes considered repair genes such as BRCA) is less common.

When CUN often occurs intestinal form of gastric cancer by Lauren classification, with GS (genetically stable variant) - diffuse type of gastric cancer. It is believed that due to low concentration or defectiveness of "intercellular cement" E-cadherin in diffuse gastric cancer comes a fast hematogenous dissemination. MSI is associated with hypermethylation [42, 43] of the most active regions of DNA - CpG-islands (spots where the cytosine is preceded by a guanine). There are some reports about the greater sensitivity of this genetic categories to irinotecan-based chemotherapy regimen. EBV-cancer most often affects the antral part of stomach.

Genetic analysis of MSI alone seems to be used as a surgical predicting factor, because classical chemotherapy, particularly in elderly patients, in the case of MSI isn't promising [50]. This type of tumors exhibit a local pronounced immunosuppressive activity [48, 49]. Therefore, as EBV gastric cancer, MSI gastric cancer is considered promising for the novel immunological drugs (PD-L1 / PD-L2 namely Ipilimumab, Nivolumab, Pembrolizumab) administration [39].

Already available monoclonal antibodies for a broader study of microsatellite unstable gastric cancer patients [57]:

Mismatch Repair Protein (MLH1) - antibody ES05
Mismatch Repair Protein (MSH2) - antibody 25D12
Mismatch Repair Protein (MSH6) - antibodies PU29
Mismatch Repair Protein (PMS2) - antibodies M0R4G.

Imyanitov et al. [50] comments on the importance of MSI analysis to chemotherapy and its particular importance for surgical oncology:

1. Most of the microsatellite-unstable carcinomas (MSI-H colorectal cancer, gastric cancer, ER belong to Lynch syndrome) are sporadic. [51, 52].

2. Hereditary colorectal cancer (CRC) affect relatively young subjects, the sporadic cases of MSI-H (H - high, high level of volatility) accumulate in elderly patients.

Hereditary cancers occur because of mutational inactivation MLH1, MSH2, PMS2 or MSH6 whereas sporadic MSI-H tumors typically arise as a result of gene promoter methylation of MLH1, which may be due to widespread abuses, epigenetic regulation ("methylator phenotype"). For some

unknown reason, the BRAF mutations occur only in sporadic, but not hereditary MSI-H tumors [52, 53].

3. Although the MSI-H tumors are undifferentiated, they are usually characterized by occurrence of the disease. Specifically, MSI-H tumors show a relatively low rate of recurrence after surgery [54, 55]; accordingly, only 4% of locally advanced colorectal cancer have MSI-H phenotype [56, 58]. As a result, cases of MSI-H are extremely rare in the research related to metastatic cancer, so a direct clinical assessment of chemo-sensitivity is very complex. Most of the data on response to treatment for cases of MSI-H is derived from adjuvant therapy, where a reliable prognostic value of this parameter is not always possible.

4. Sensitivity MMR-deficient cells to various anticancer drugs is the subject of numerous laboratory studies. It is important the natural cancer cells with MSI-H have an exceptionally high mutation rate, and therefore accumulates a considerable amount of "secondary" genetic lesions; depending on the spectrum of the target genes, the secondary damage may significantly alter the response to therapy [56, 59]. Furthermore, MMR inactivation of various genes, for example, MSH2 and MLH1, can lead to various chemosensitivity models [62].

5. Most cases of preclinical studies indicate resistance MSI-H cell to 5-fluorouracil (5-FU) [56, 63]. MSI-H status is also associated with low sensitivity to Cisplatin, Carboplatin, 6-thioguanine, however, these compounds aren't used in any way used for the treatment of colorectal cancers [56, 61-63]. Whereas MMR-deficiency associated with insensitivity to Cisplatin [64, 65, 66] and Carboplatin, Oxaliplatin. Several studies have shown particular sensitivity of MSI-H cells to Irinotecan; It was shown that the response to a topoisomerase I may be mediated by the presence of secondary mutations in the genes and MRE11 Rad50 [61]. Methotrexate is a selective inhibitor of the MSH2-deficient cells; MLH1-defective cells showed particular sensitivity to this compound [60, 62].

6. There is a consensus in the literature that the MSI-H patients do not use 5-fluorouracil-based adjuvant therapy [67]. Some reports suggest even worse result compared with patients treated; PCTs may endanger the natural immune response to MSI-H cells [56, 68, 69]. Adjuvant chemotherapy in patients with hereditary colorectal cancer cases also showed no benefit from 5-FU [70]. Given the improved outlook MSI-H tumors, reputedly, adjuvant therapy *should be omitted* for the MSI-H colorectal cancer [56, 71, 72]. The combination of 5-FU with Oxaliplatin has been recently included in guidelines for adjuvant treatment of stage III colorectal cancer; since only a few MSI-H patients are currently available, it is still not possible to draw conclusions from existing data sets [73, 74]. Experiments with Irinotecan did not qualify this product for use in adjuvant therapy. However, analysis of a subgroup of patients with MSI-H showed that this particular category of patients may benefit from the addition of Irinotecan, Leucovorin and 5-Fluorouracil [75].

7. Data on the use of chemotherapy for common MSI-H cancers are limited to small studies. Liang et al. [76] and Brueckl et al. [77] reported an improvement in the response of microsatellite unstable colorectal cancer to 5-fluorouracil. There are contradicting informations about the role of MSI status in determining the response to the combination therapy of 5-FU and Oxaliplatin [78-80]. Several reports have shown a greater incidence of MSI-H response to Irinotecan [81-83], although this claim has been challenged by a recent study, Kim et al. [84].

Given the above, MSI-H cancers correspond fairly uncommon clinical situation, expressed by the words "big tumor without distant metastases" (As opposed to when multiple distant metastases manifested, however primary tumor cannot be found at all). These cancers have the worst indicators of aggressive histology. They are poorly differentiated, have inf-γ microscopic type of tumor infiltration, invade surrounding structures and can have multiple metastases in regional lymph nodes. Thus, after their removal, sufficient local, regional and overall result is affordable.

Another, identified recently, genetic feature is the membership of the GC patients (less than 5%) to the so-called "Angelina Jolie syndrome" [37]: genetically transferred mutations of one of the alleles of the BRCA-1 gene. Other diseases, related to this syndrome is breast cancer and ovarian cancer. Deletion of the remaining healthy allele (LOH - loss of heterosigosity, loss of heterozygosity) leads to the second type of hereditary gastric cancer (first - CDH1-associated). Because all hereditary cancers are dominantly inherited, this mutation appears at a relatively early age (before age 50).

Also, as for the BRCA-associated breast cancer, the presence of the gene amplification means when RJ resistance to chemotherapeutic regimens based Taxanes [35] and Anthracyclines and sensitivity to Mitomycin C [34], Cisplatin and PARP-inhibitors (poly-ADP ribose polymerase inhibitors: PARIBAS [33]), the prospect of immunotherapy [40]. Her-2neu is positioned not only as a sensitivity factor to Trastuzumab therapy, therapy and sensitivity Epirubicin, Doxorubicin [25] liposomal doskorubitsinom.

All of the above means the prospects of this trend analysis is not even in the laboratory and the field, the clinical setting.

Immunohistochemical studies were performed to assess the expression of p53 protein products, VEGFR-3, Ki67 [6], Her2 \ new (Figure №4 and №5). One of the most important fact about the expression of Her2 \ new in stomach cancer is a lenient approach for the recognition of the marker positive, compared with breast cancer [30]. In our study, "++" marker was considered positive. While criterion "+++" is indispensable for breast cancer.

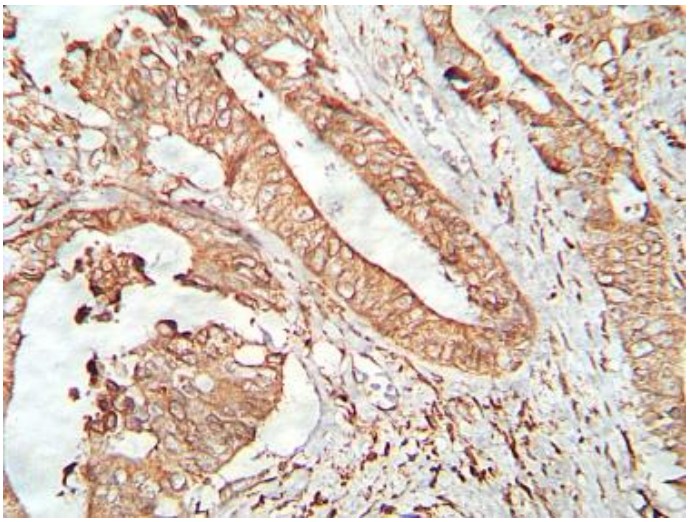

Fig. 4. Photomicrograph. Study of the expression of Her-2neu gene (with protein-erB-2 Oncoprotein): «+++».

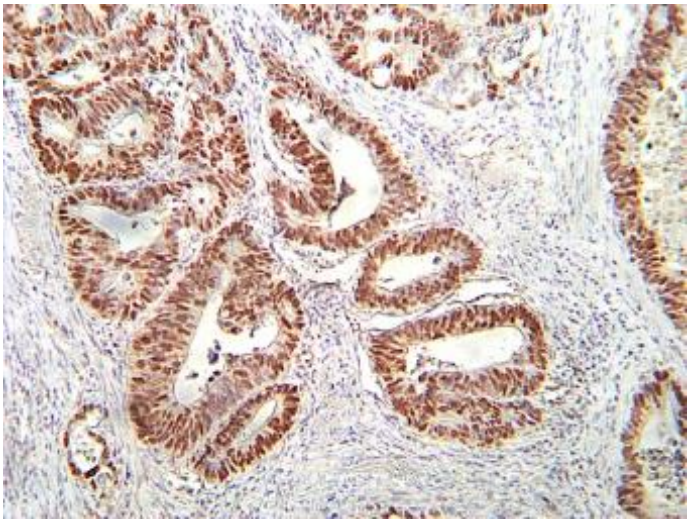

Fig. 5. Fig-. Tissue image with the expression level of Ki67 Protein (MIB-1) - 80%.

Ki67 - nuclear antigen, antibodies stain on which there arein case cells in the proliferative phase of the cell cycle (G1, S, G2, M). It is also considered mitotic index. Penault-Llorca established [19] that patients whose tumors express the Ki67 more than 50% of the cells have a significant recurrence of the disease (not local, namely clinical recurrence). Ki67 expression study associated with sensitivity to Docetaxel [19]. In our study, it was considered positive expression in more than 30% of the cells. Reagent used - MIB-1 monoclonal antibody.

VEGFR-3 and its sprout factor VEGF-C is associated with a very poor prognosis in gastric cancer [21]. Finally, VEGFR-3 is not a usual marker of the defective neoangiogenesis, which occurs in the tumor (vascular education without pericytes, endothelial cells only). VEGFR, as well as Ki67 can be used as a prediction factor for therapy: Bevacizumab assignment does not require execution of the analysis [20]. VEGFR-3 (VEGF-C) is a factor that can be used to analyze the

process of formation of new lymphatic vessels in the tumor itself and around it - because called lymphangiogenesis [23, 24]. In a normal situation VEGFR3 endothelium expressed early embryos.

Honestly speaking, all the microscopic blood vessels in a tumor can be divided into two groups namely;

1- The slit formation with a very thin wall

2- 2-classical microscopic vessels.

The first are microscopic lymph capillaries and contains lymph , second - blood capillaries (see Figures 6 and 7.). VEGFR3 Marker (see. Figure 8) related to the first lymph capillaries. It was also noted that the expression of these tumor markers is inversely proportional to the degree of dissemination through the blood capillaries. The tumors also had a compensatory mechanism whereby the tumor "turned" to create new lymphatic capillaries, through which it could spread in a process called dissemination .Failure of the lymphogenic dissemination the tumor turned into a hematogenous dissemination spreading through the blood capillaries. The presence of circulating tumor cells in the bloodstream does not mean directly the presence of hematogenous metastasis, but is a factor of poor prognosis [45, 46]. The properties of tumor microvessels can determine the effectiveness of a particular type of chemotherapy [47].

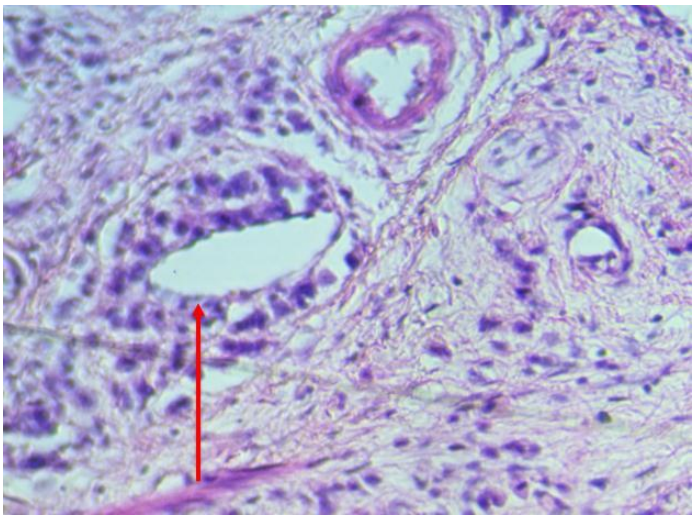

Fig. 6. Photomicrograph. Microscopic cracks unlike lymph and capillary vessels (at the top of the photo is visible the microscopic capillary with pronounced muscular component of the wall, just below - pronounced perivascular growth).

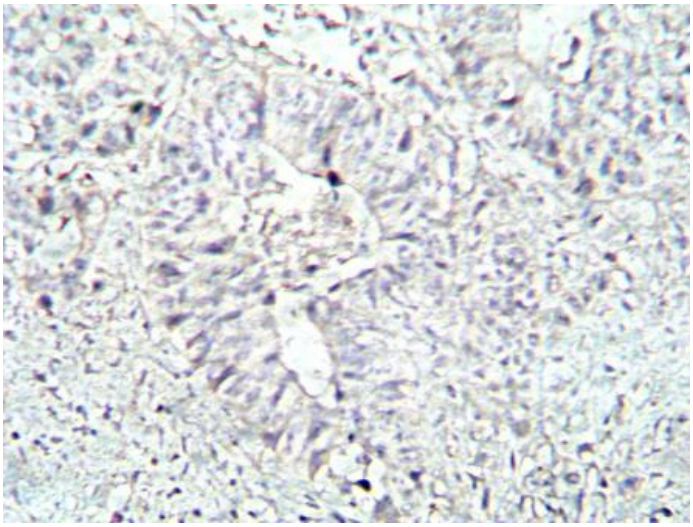

Fig. 7. Photomicrograph. Vascular Endothelial Growth Factor Receptor (VEGFR-3) (KLT 9) +

Promising, but not included in our study, the marker is Her1 or, as it is called today EGFR. We have one observation RJ mutation of the gene and expression of the corresponding protein product.

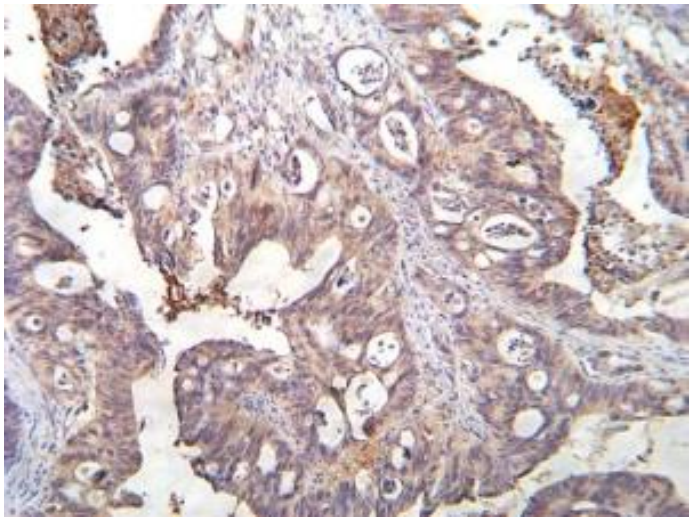

Fig. 8. The photomicrograph. Immunohistochemical stains for Epidermal Growth Factor Receptor (EGFR) (HER-1), the expression level of "+".

Materials and methods

When analyzing the data via PubMed and Google Scholar search engine has received the following number of searches:

`Gastric cancer her2 / new` - 165 (189) Links

`Gastric cancer p53` - 2102 (210 000) links

`Gastric cancer VEGFR` - 263 (19800) links

`Gastric cancer VEGF-C` - 137 (9960) references

`Gastric cancer Ki-67` - 733 (31300) links

`Gastric cancer p 53 Ki-67 VEGF` - 1 (6730) link

`Gastric cancer p 53 Her2 VEGF` - 2 (43700) links

`Gastric cancer p 53 Her2 ki-67` - 2 (4620) Links

`Gastric cancer Her2 ki-67 VEGF` - 0 (2200)

`Gastric cancer Her2 p53 ki-67 VEGF` - 0 (1740)

`Gastric cancer Her2 / new p53 ki67 VEGF-C` - 0 (0) No

In parentheses is the number of links on the subject on Google Scholar system.

Thus, the selected combination of IHC markers in the study entitled "stomach cancer" seems to be original and at the same time, relevant. No studies indicated in the search PubMed and Google Scholar systems to include all 4 of these histochemical criterion for gastric cancer (Table 1).

Table 1. Combination of two or more factors on PubMed search

gastric cancer	her2/new	p53	VEGF	Ki-67
her2/new	-	23	38	10
p53	23	-	60	206
VEGF	38	60	-	33
Ki-67	10	206	33	-

Paraffin blocks were used for immunohistochemical staining VEGF-C (KLT reagent 9). Thin sections were prepared, which were treated with a solution of 0.3% hydrogen peroxide for 10 minutes at room temperature. For antigen detection, the sections were treated with sodium citrate pH 6.0 and placed in a microwave oven. During 12 hours at 4 ° C in humid conditions we observed processing of primary antibodies: goat polyclonal VEGF-C antibody [1: 100, Biotechnology Santa Cruz, Santa USA]. Then the sections were washed three times in phosphate buffer solution for 2 minutes, for 30 minutes at room temperature the solution was placed in horse reddish peroxidase (Envision, DAKO), labeled goat antibodies. Was added 3,3-diaminobenzidine. Normal goat IGG served as a negative control for the detection of VEGF-C. The degree of staining intensity was classified into four degrees: none (0), mild (1), moderate (2) and severe (3).

Immunohistochemical staining procedure for the study of other markers differ almost only in the form of antibodies used: for the p53 - is a Do-7, m7001, DAKO, Glostrup, Denmark, for Her2 / new - it is a 2-erB-Oncoprotein, Ki-67 - reagent MIB- 1.

Although to fully genetically classify this group of patients (188 patients radically operated), it's not enough data, of principal interest here is the relationship of histology and immunohistochemistry RJ.

When assayed Chi-square relationship of the following characteristics were studied, resulting in a pair of histological / immunohistochemical (T / S) RJ structure. The main aim of the study was to monitor the trends, the ability to predict the value of the test in terms of the other, as well as mathematical modeling of the ability to use a new marker for clinical needs. "Necessity for the doctor is the exact mathematical value which intends looking forward it's PLR".

To evaluate the results obtained using standard descriptive statistical markers and evaluation of diagnostic tests:

OR

Sensitivity

SpecificityPPV

NPV

PLR

NLR

OR - Odds ratio, is used to quantitatively describe how the presence or absence of the characteristic X correlated with the presence or absence of certain properties in the Z group. It's of vital importance, if the OR is greater than 1, this relationship is positive. In biological studies OR rarely exceeds 10. As will be shown below, in our study a number of superiority OR 10 several times. For example, when it comes to the relationship between the value of the diagnostic VEGFR-3 protein and tumor invasion into microscopic vessels, OR-10 OR exceeded a hundred times.

Sensitivity – It's the proportion of positive predictor among those where it is really positive. For example, if the sensitivity of a diagnostic marker of 90% in 9/10 patients it will be changed (increased).

Specificity – It's the fraction of the normal value of the marker in an environment where it's really normal. For example, if 80% specificity, in healthy patients marker 8.10 (diagnostic test) is normal. Both markers are considered as representing a diagnostic value if they represent a specificity greater than 75%.

Using the following markers is a relatively recent innovation in medical research. If you make a selection of scientific works 10-20 years ago, these markers are usually not mentioned in them.

PPV - Positive predictive value: positive predictive role or ability to predict a positive test result correctly. This is the proportion of patients with positive test results who were correctly diagnosed using the test diagnostic system.

NPV - Negative predictive value: negative predictive weight, the ability to predict a negative test result correct; the proportion of patients with negative test results, which investigated the diagnostic system (right) delivered a negative result.

How to interpret the values obtained in the studies of these parameters. There is no clear answer in the literature can be found. However, one look at the interpretation of other similar criterion - the famous Pearson correlation coefficient [32] - has a significant response to these concerns.

0.2 Very weak correlation

0.2 - 0.5 A weak correlation

0.5 - 0.7 Average correlation

0.7 - 0.9 High correlation

0,9 very high correlation

However, it is much harder for the next group of evaluation criteria: PLR and NLR (See Table 2).

Table 2. Mathematical interpretation of growth positive likelihood ratio.

Likelihood Ratio	**Estimated change in [2] probability (%) ***
Values between 0 and 1 decrease the likelihood of having the disease	
0.1	−45
0.2	−30
0.3	−25
0.4	−20
0.5	−15
1	0
Values greater than 1 increases the probability of having the disease	
2	+15
3	+20
4	+25
5	+30
6	+35
7	
8	+40
9	
10	+45

PLR - Positive likelihood ratio; the likelihood ratio of a positive test result. It is believed that if a likelihood ratio test of> 1, then the positive test is associated with the disease. If the likelihood ratio <1 - a positive result is associated with the absence of disease. In interpreting the plain language, PLR - it is mathematically predicted the diagnostic value of the newly discovered marker for clinical needs.

NLR - Negative likelihood ratio; negative likelihood ratio test result. Negative diagnostic accuracy of the test. The lower the figure, the probability of a correct diagnosis of exclusion increases.

Both of these indicators are directly related to the ability to predict the results of the test, i.e, importance of it for future clinical use, the importance in clinical practice.

For example, the same early mentioned VEGFR-3 is the indicator "for further diagnostic clinical value» PLR = 89,333 with CI95% 15,867-1728,203, when it comes to its ability to "predict" the degree of hematogenous dissemination RJ.

This relationship is reversed: the higher the (neo) lymphangiogenesis, the less there is hematogenous dissemination through the blood capillaries (Vo-V3) and vice versa. The lower (neo) lymphangiogenesis, the more tumor emboli in the capillaries (see. Table 5).

Clinical conclusion may be, for example, keeping the concentration of VEGFR-3 oncoprotein in gastrobioptatov? as one of the factors of preoperative planning vastness remove lymphatic nodes during operations on the stomach. Along with such important factors as the age of patients, the presence of severe co-morbidities, the degree of histological differentiation (there are reports that at signet cell cancer D2 less effective), already diagnosted hematogenous metastases (liver, ovaries), clinics installation, socio-economic constraints and so on.

Calculations were made using mathematical power of online calculators available on the website http://statpages.info/. The results, depending on the level of accuracy to classify gray, blue and red zones, as shown in the table. The gray area - the result insignificant in terms of the further introduction into clinical practice, the blue zone - a significant result from a mathematical point of view, the red zone - a high-level results. For example, the odds ratio <1 is uninformative,> 1 informative >> 1 highly informative. As can be seen from the attached table, highly informative results were not uncommon.

Study proportion of patients with different tumor differentiation among patients with gastric cancer with different expression of IHC markers.

Distribution of patients with gastric cancer expression of various factors was interesting from the point of view of expression of a marker in the group. p53 + (49,52%), VEGFR-3 + (43,56%), Ki67 + (33,33%), Her2 \ new + (62,22%) patients had a high and a moderate degree of histological differentiation from all IHC tests had positive expression. The high level erbB2

specifically for this sample of patients had a synonymous less aggressive course; in contrast to the other three indicators. Almost the same as that for breast cancer: Her2 \ new +, in contrast to the triple negative cancers, was nevertheless more favorable flow form (Her2, a genetic form of breast cancer, along with the luminal A and B types).

Counting was carried out not from all 188 patients, but only on all the positive, and then by all the negative. p53 (22,89%), VEGFR-3- (35,63%), Ki67- (65,79%), Her2 \ new- (34,96%) patients had a high and a moderate degree of histological differentiation from all IHC tests, had no expression at all (see. table 3).

Table 3. Study of histological and immunohistochemical markers in the forecast RJ: the degree of differentiation.

		p53			VEGFR-3			**Ki67**			**Her2\new**		
		+		-	+		-	+		-	+		-
	g1g2	52		19	44		31	50		25	28		50
	g3g4	53		64	57		56	100		13	17		93
p		**p<0,0001**			p=0,298			**p<0,0001**			**p=0,002**		
		n	CI 95%		n	CI 95%		N	CI 95%		n	CI 95%	
OR		**3,305**	**1,667**	**6,600**	1,394	0,742	2,626	0,260	0,114	0,585	**3,064**	**1,452**	**6,509**
Чувстви-тельность		0,495	0,430	0,552	0,436	0,366	0,503	0,333	0,300	0,372	0,622	0,484	0,746
Специфич-ность		**0,771**	0,688	**0,843**	0,644	0,563	0,722	0,342	0,211	0,496	0,650	0,607	0,689
PPV		0,732	0,636	**0,816**	0,586	0,493	0,677	0,667	0,600	0,745	0,359	0,280	0,430
NPV		0,547	0,488	0,538	0,496	0,433	0,556	0,115	0,071	0,167	**0,845**	**0,789**	**0,896**
PLR		2,163	1,380	3,510	1,223	0,836	1,808	0,507	0,380	0,739	1,780	1,233	2,400
NLR		0,655	0,532	0,828	0,877	0,689	1,127	1,949	1,265	3,323	0,581	0,369	0,849

Research sometimes involves the fact that some results can not be explained at all, or not enough data. For example, it is clear why the mitotic index in most of the g1 + g2 patients was less than 30. These tumors are divide slowly, slower progress are ts more "benign". Obviously, because of the low potential of aggression, patients with VEGFR-3-positive tumors was less here. The same applies to another "genetic instability factor" - the protein p53.

p53 + (50,48%), VEGFR-3 + (56,43%), Ki67 + (66,67%), Her2 \ new + (37,78%) patients had a low degree of or no histological differentiation from all the IHC test at all and at the sametime had positive expression being a clear indication of poor prognosis in clinical practice. More aggressive poorly differentiated tumors had accumulated large numbers of p53 proteins, VEGFR-3

and had much worse indicators of mitotic activity. IHC analysis is not genetic, and it is impossible to judge the extent of such a mutant p53, and about which the mutations in question. Immunohistochemistry gives only indirect information. If p53 protein becomes much, it means there is an increased expression of TP53 gene. This could be used, along with conventional histological markers.

p53 (77,11%), VEGFR-3- (64,37%), Ki67- (34,21%), Her2 \ new- (65,04%) patients had a low degree of or no histological differentiation from all IHC tests, had no expression at all. Of the most impressive group of negative values of negative markers made that the absence of erbB2 coincided with the malignancy of the tumor.

RESEARCH AMONG g1g2 and g3g4 ONCOPROTEINS.

Total interest structure of each combination of markers among patients with different tumor differentiation in a number of cases in need of interpretation. Because here the ratio produced by all 188 patients, and not separately from "positive" and individually a "negative", as it was done in the above. This was necessary in order to see the TS "General background" on which all the events occurred.

Table 4. Study of histological and immunohistochemical markers in the forecast RJ: aggressive local growth.

	p53 T			**VEGFR-3 T**			**Ki67 T**			**Her2\new**		
	+	-		+	-		+	-		+	-	
T1, T2	30	0		19	18		37	0		0	35	
T3	19	0		0	20		25	0		13	14	
T4	64	75		72	59		75	51		36	90	
Σ	113	75		91	97		137	51		49	139	
p	**p<0,0001**			**p<0,0001**			**p<0,0001**			**p<0,001**		
	n	CI 95%		n	CI 95%		n	CI 95%		n	CI 95%	
OR	27,108	3,803	547,425	21,111	2,495	466,305	18,870	2,655	380,367	0,066	0,003	0,473
Чувствии-тельн.	0,265	0,226	0,274	0,950	0,768	0,997	0,270	0,238	0,277	-0,20	0,001	0,109
Специфичность	0,987	0,929	0,999	0,526	0,430	0,551	0,981	0,895	0,999	0,765	0,759	0,795
PPV	0,968	0,825	0,998	0,514	0,415	0,539	0,974	0,856	0,990	0,028	0,001	0,151
NPV	0,475	0,447	0,481	0,952	0,779	0,997	0,338	0,308	0,344	0,699	0,694	0,727
PLR	20,177	3,168	397,774	2,006	1,347	2,223	14,044	14,044	275,279	0,085	0,004	0,53
NLR	0,744	0,727	0,833	0,095	0,005	0,540	0,744	0,744	0,852	-	-	-

Vascular VEGFR-3 protein showed a positive reaction (due to the rarity of its expression level was considered positive, since 1+) g3g4 patients at 30.32%, 29.79% value almost coincided with the group, such as where it patients It was negative. A negative result - it is also a result. In the group with all the mitotic index were much more optimistic: the leader of Ki67 + g3g4 differed from the nearest pursuer, more than doubled, with the result of 53.19% of the patients. Almost the same thing happened to a group of Her2 \ new- g3g4 49,47%.

Study proportion of patients with varying degrees of swelling of the stomach wall sprouting among patients with gastric cancer with different expression of IHC markers.

Theoretically, p53, and VEGFR-3 could be used to increase the chance of performing more advanced operations in a long list of others, certainly more important the surgical criteria, such as infiltrative tumor growth (see. Table 7), "bad" histology (G3, G4), young age, and others. As we have seen above, the PLR value = 20.177 giving more than 45% increase in the probability of performing the combined operation, with an increase in the concentration of tumor p53 protein product. The same (14.044) is also valid in relation to factor Ki67 proliferation.

Table 5. Study of histological and immunohistochemical markers in the forecast RJ: tumor emboli and invasion into the blood microvessels.

	p53 V		**VEGFR-3 V**		**Ki67 V**		**Her2\new**	
	+	-	+	-	+	-	+	-
Vo	52	9	67	0	88	0	13	57
V1	9	8	0	27	0	13	0	24
V2	20	41	14	26	50	12	12	46
V3	31	18	0	54	12	13	13	23
Σ	112	76	81	107	150	38	38	150
p	**p<0,0001**		**p<0,0001**		**p<0,0001**		p=0,711	

	n	CI 95%		N	CI 95%		n	CI 95%		n	CI 95%	
OR	**6,452**	**2,776**	**15,411**	**512,071**	**66,910**	**10719,582**	**53,935**	**7,619**	**1084,591**	0,848	0,376	1,899
Чувствит.	**0,464**	**0,409**	**0,503**	**0,827**	**0,774**	**0,839**	**0,587**	**0,560**	**0,587**	0,342	0,209	0,496
Специф.	**0,882**	**0,800**	**0,938**	**0,991**	**0,951**	**1,000**	**1,000**	**0,893**	**1,000**	0,620	0,586	0,659
PPV	**0,852**	**0,751**	**0,923**	**0,985**	**0,922**	**0,999**	**1,000**	**0,954**	**1,000**	0,186	0,114	0,269
NPV	**0,528**	**0,479**	**0,562**	**0,884**	**0,849**	**0,892**	**0,380**	**0,339**	**0,380**	0,788	0,745	0,838
PLR	**3,921**	**2,049**	**8,164**	**89,333**	**15,867**	**1728,203**	**22,88**	**3,935**	**442,038**	0,900	0,506	1,453
NLR	**0,608**	**0,530**	**0,738**	**0,174**	**0,161**	**0,237**	**0,424**	**0,408**	**0,516**	1,061	0,765	1,348

In addition to determining the degree of differentiation of the primary tumor, the tumor incidence in the stomach, sprouting surrounding structure and so forth., Investigated lymph nodes by the number of groups № 1-16, the presence of tumor emboli in the capillary bed (V), the presence of residual tumor tissue (R) (cm., table 6).

Table 6. Study of histological and immunohistochemical markers in the forecast RJ: residual residual tumor disease.

		p53			**VEGFR-3 R**			**Ki67 R**			**Her2\new**		
		+	-		+	-		+	-		+	-	
	R-	96	74		98	10		141	28		42	129	
	R+	9	9		3	17		3	16		8	9	
p		p=0,626			**p<0,0001**			**p<0,0001**			**p=0,045**		
		n	CI 95%		n	CI 95%		n	CI 95%		n	CI 95%	
OR		**1,297**	0,445	3,781	**7,933**	**2,080**	**35,505**	**26,875**	**6,709**	**125,108**	0,366	6,120	1,125
Чувствит.		**0,914**	**0,876**	**0,953**	**0,970**	**0,925**	**0,992**	**0,979**	**0,949**	**0,994**	**0,840**	**0,760**	**0,917**
Специи-фич.		0,108	0,059	0,157	0,195	0,143	0,221	0,364	0,265	0,413	0,065	0,036	0,093
PPV		0,565	0,541	0,589	0,583	0,556	0,596	**0,834**	**0,809**	**0,847**	0,246	0,222	0,268
NPV		0,500	0,274	0,726	**0,850**	**0,624**	**0,960**	**0,842**	**0,614**	**0,958**	0,529	0,295	0,754
PLR		1,025	0,931	1,131	1,206	1,080	1,273	1,539	1,291	1,695	0,899	0,789	1,010
NLR		0,790	0,299	2,091	0,152	0,036	0,520	**0,057**	**0,014**	**0,192**	2,453	0,898	6,593

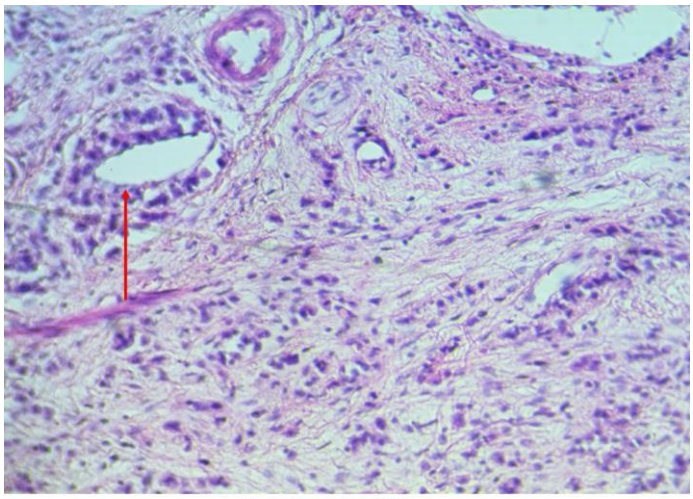

Fig. 9. "Annular" enveloping microscopic vascular tumor tissue (arrow).

The degree of vascular involvement was classified according JRSGC [31]:

v0 - no vascular invasion;

v1 - the minimal vascular invasion;

v2 - moderate vascular invasion;

v3 - severe (severe) vascular invasion (see Figure. 9).

Table 7. Study of histological and immunohistochemical markers in the forecast RJ: RJ type of infiltrating activities.

		p53		VEGFR-3 Inf α, β Inf ϒ		Ki67 Inf α, β Inf ϒ		Her2\new	
		+	-	+	-	+	-	+	-
	Inf α, β	75	41	79	27	122	10	25	90
	Inf ϒ	30	42	28	54	34	22	26	47
p		**p=0,002**		**p<0,0001**		**p<0,0001**		**p=0,044**	
		n	CI 95%	n	CI 95%	n	CI 95%	n	CI 95%
OR		**2,561**	**1,341** **4,907**	**5,643**	**2,865** **11,191**	**7,894**	**3,183** **19,954**	0,502	0,248 1,014
Sensitivity.		0,714	0,648 **0,777**	0,738	0,674 **0,795**	0,782	0,747 0,810	0,490	0,369 0,614
Specificity.		0,506	0,422 0,585	0,687	0,581 0,742	0,688	0,518 0,824	0,343	0,298 0,389
PPV		0,647	0,586 0,703	0,745	0,680 **0,803**	**0,924**	**0,883** **0,957**	0,217	0,164 0,272
NPV		0,583	0,486 0,674	0,659	0,574 0,733	0,393	0,296 0,471	0,644	0,559 0,730
PLR		1,446	1,120 1,872	2,215	1,609 3,085	2,503	1,552 4,601	0,746	0,526 1,003
NLR		0,565	0,382 0,835	0,393	0,276 0,561	0,317	0,231 0,487	1,486	0,992 2,118

The red color in the table marked with numbers, outstanding in terms of medical statistics biological research. For example, the odds ratio (the OR), equal to 27.108 (with a confidence interval of 3,803-547,425) for p53 and 21.111 (with a confidence interval= 2,495-466,305) is more characteristic of the mathematical accuracy of physical and engineering sciences. Significant indicators of sensitivity T / VEGFR-3, and the specificity of the T / p53, as well as indicators of T / p53 / the PPV, T / VEGFR-3 / the NPV, T / Ki67 / PLR consistent high quality of the study. At the same practical significance of the data "super-precision" dubious: it would be possible to try to predict the rate of tumor growth according to the criterion of T for different concentrations of oncoproteins, but it does not have enough data (see table 4.).

The percentage of patients in the study had exponents ralichnye sprouting tumor gastric wall: p53 + (43,36%), VEGFR-3 + (20,88%), Ki67 + (27,00%), Her2 \ new + (0%) - during germination of the mucosa and submucosal tumor. p53 (0%), VEGFR-3- (39,18%), Ki67- (0%), Her2 \ new- (25,18%) - during germination mucosa and submucosa tumor. p53 + (56,64%),

VEGFR-3 + (79,12%), Ki67 + (73,00%), Her2 \ new + (100%) - germination serous membrane swelling and sprouting in adjacent organs (poor prognosis). p53 (100%), VEGFR-3- (60,82%), Ki67- (100%), Her2 \ new- (74,82%) - serosa tumor germination and sprouting into surrounding organs. In general, the faster the growth characteristic mestnodestruiruyuschy tumors with mutant p53 much greater mitotic index and high tendency to form microscopic vessels.

Table 8. Study of histological and immunohistochemical markers in the forecast RJ: the presence of metastases in regional lymph nodes.

		p53		**VEGFR-3**		**Ki67**		**Her2\new**	
		+	-	+	-	+	-	+	-
	No	55	22	55	29	11	17	26	58
	N1	22	23	42	15	42	19	17	27
	N2	22	44	16	28	43	29	15	39
	N3	0	0	1	2	20	7	0	6
Σ		99	89	117	71	116	72	58	130
p		**p<0,0001**		p=0,234		p=0,011		p=1,000	

	n	CI 95%		n	CI 95%		n	CI 95%		n	CI 95%	
OR	**3,807**	**1,952**	**7,472**	**1,447**	0,766	2,738	0,339	0,137	0,830	1,009	0,516	1,969
Чувствит.	0,556	0,485	0,618	0,482	0,421	0,541	0,095	0,055	0,140	0,448	0,337	0,563
Специфит.	**0,753**	**0,674**	**0,822**	0,608	0,513	0,699	**0,764**	**0,700**	**0,836**	0,554	0,504	0,605
PPV	0,714	0,624	0,794	0,655	0,571	0,735	0,393	0,229	0,579	0,310	0,233	0,388
NPV	0,604	0,541	0,659	0,433	0,365	0,497	0,344	0,315	0,376	0,692	0,630	**0,756**
PLR	2,247	1,490	3,473	1,231	0,864	1,797	0,402	0,185	0,853	1,005	0,679	1,424
NLR	0,590	0,465	0,763	0,851	0,656	1,129	1,185	1,029	1,349	0,996	0,723	1,315

Germination throughout the T4 wall compared with earlier stages T1-T3. A glance at the table was enough to understand that oncoproteins concentration in locally advanced tumors were larger than in earlier. Perhaps this is due to the longer duration of the existence of a larger tumor, the accumulation of factors of aggression is slow. One is struck by the presence of the results of the "red zone": for example, the odds ratio >> 1, the positive likelihood ratio >> 1.

The following table (Table 9) are marked in gray are mathematically "optimistic" markers of progression, which had, in our view, no practical application. They did not affect the frequency of microscopic residual tumor disease, metastasis to the lymph nodes (see. The data reflected in Table 8), and the degree and type of infiltration of the gastric wall were more dependent on other factors. Black abandoned those relations, whose clinical significance is questionable. And finally, a dark red

- that can be used in planning further studies, with more advanced molecular genetic tests (MSI) inclusive.

Table 9. Search diagnostically promising among the "optimistic" of pairs of markers.

OR	SENS	SPEC	PPV	PLR	NLR
T/p53 27,108(3,803-547,425)	**VEGFR-3/T 0,952(0,779-0,997)**	VEGFR-3/V 0,991(0,951-1,000)	VEGFR-3/V 0,985(0,922-0,999)	**Ki67/T 14,044(14,044-275,279)**	**T/VEGF 0,952(0,779-0,997)**
VEGFR-3/Inf 5,643(2,865-11,191)	**VEGFR-3/T 0,950(0,768-0,997)**	Ki67/V 1,000(0,893-1,000)	Ki67/V 1,000(0,954-1,000)	VEGFR-3/V 89,333(15,867-1728,203)	
p53/V 6,452(2,776-15,411)	**VEGFR-3/R 0,970(0,925-0,992)**	**p53/T 0,987(0,929-0,999)**	**PPV/p53/T 0,968(0,825-0,998)**	Ki67/V/PLR 22,88 (3,935-442,038)	
VEGFR-3/T 21,111(2,495-466,305)	**T/VEGF 0,950(0,768-0,99)**	**T /p53 0,987(0,929-0,99)**	**T/p53 0,968(0,825-0,998)**	**p53/T 20,177(3,168-397,774)**	
VEGFR-3/V 512,071(66,910-10719, 582)	**Ki67/R 0,979(0,949-0,994)**			**T/p53 20,177(3,168-397,774)**	
p53/T 27,108(3,803-547,425)				**T/Ki67 14,044(14,044-275,279)**	
Ki67/Inf 7,894(3,183-19,954)					
Ki67/V 53,935(7,619-1084,591)					
Ki67/R 26,875(6,709-125,108)					
VEGFR-3/R 7,933(2,080-35,505)					
T/VEGF 21,111(2,495-466,305)					

Tracked trends in availability of oncogenetic markers and their importance in making a fresh sense of perspective for further development of oncological sciences. The abundance of available monthly appearing experimentals, practical, research results may be in the updated health information flow. Deepening practical oncology at the genetic, molecular sphere only briefly looks distracted from the realities of life scholasticism; immediately bringing new, more effective tools in the fight for the lives of patients in routine clinical practice. Exit "armchair science" in people is an event, useful for practicing oncologist and the patient.

Conclusions

1. Modern rapid molecular genetic development of cancer science creates the preconditions for a more personal effect on the tumor.

2. Generally, immunohistochemical and genetic data obtained when examining patients, sooner or later will result in a better understanding of the biology of gastric cancer, including planning of surgery in individual patients.

3. Increase the availability of genome-sequencing to the next (3-5 years) time frame will lead the study of the genetics of cancer of the stomach and the individualization of treatment to a new level.

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