The immunohistochemical profile of the adenocarcinoma of upper gastric pole

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Abstract

Although gastric adenocarcinoma continue to be the second cause of death worldwide, its incidence and mortality appear to have decreased in recent decades. Despite this decline, adenocarcinomas from proximal stomach tend to be more frequent during the last three decades. Adenocarcinomas with this location seems that are a different, specific subtype of gastric carcinoma. The purpose of this study was to clarify the differences between gastric adenocarcinomas from upper and distal gastric pole using the immunohistochemistry. For this reason, we investigate histopathological and immunohistochemically 77 cases of upper gastric pole adenocarcinoma selected from a number of 472 gastric tumors. The immunohistochemistry was performing only in 32 cases by ABC technique with the following primary antibodies: Cytokeratin 7, Cytokeratin 19, Epithelial Membrane Antigen (EMA), Carcinoembryonic Antigen (CEA), Lysozyme, Vimentin, p53 protein, CD34 and Ki67 antigen. The acquired results do not distinguish a peculiar immunohistochemically profile unlike distal gastric adenocarcinomas. Nevertheless, we pointed out the predominance of diffuse adenocarcinomas type according to Laurens classification, which immunohistochimically were strong positive to cytokeratins, EMA, CEA and lysozyme. Moreover, investigation of some antigens like lysozyme, p53, Ki67 and CD34 seems to be useful for prognostic estimation of carcinoma with this topography.

Keywords: adenocarcinoma, upper gastric pole, immunohistochemistry.

Introduction

Even though at a world’s level it continues to be the second cause of death through cancer, lately we witnessed a tendency of decrease in incidence [1, 2]. In China, South America, Eastern Europe and Japan, gastric cancer is one of the most frequent malignant neoplasia [3].

From the two types defined by Lauren P [4], the intestinal type is the most frequently met, developing in the gastric distal region and predominant in the endemic zones. The diffuse type has a worse prognostic and tends to develop in young patients, in any gastric localization, but especially at the level of cardiа [5, 6].

During the last period, it was registered an increase of proximal gastric cancers in USA as well as in Europe and Asia [7–12], these ones having a clinical pattern and a different biologic behavior from all the other localizations. Generally, proximal gastric cancer has a bigger incidence in the white people [13], being most often associated to obesity [9, 14, 15] and chronic smoking [12, 14, 17, 18] as ethiopathogenic factors and has a worse diagnostic than the cancers of the region. The purpose of our study has been to establish the immunohistochemical profile of the cancer of upper gastric pole and to identify the potential prognostic markers for this localization of gastric cancer.

Material and methods

The study material was represented by 77 cases of adenocarcinoma of upper gastric pole, cases selected from a number of 472 gastric tumors clinically diagnosed within the period 1996–2005 in the General Surgery Clinics I, II and III of the Emergency County Hospital of Craiova. Most of the cases of adenocarcinoma of upper gastric pole (39 cases) were developed at ages between 40–65 years old, especially in male persons (57 cases), and most frequently accused disphagy (57 cases), ponderal decrease (52 cases) and abdominal pain (44 cases), and in the medium period of time passed from the beginning of the symptomatology and the moment of diagnosis was of 13 months.
The pieces of surgical resection have been sent to the Laboratory of Pathologic Anatomy of the same hospital for histopathological examination.

The macroscopic exam has shown the predominance of vegetative forms with 32 cases, followed by the diffuse infiltrative form (19 cases), ulcerate (15 cases), polypoid (nine cases) and superficial (two cases).

The histopathological interpretation has supposed the fixation of the pieces of surgical exeresis in 10% formalin solution – buffered, inclusion of paraffin, sectioning at microtome with the obtaining of histopathological sections having a 3–5 µm thickness and a staining with: Hematoxylin–Eosin (HE), trichromic Goldner–Szeckelly (tr. GS), Periodic Acid Schiff–Hematoxylin Mayer (PAS–HE), Blue Alcian–Periodic Acid Schiff (AA–PAS), Blue Alcian (pH 0.2; pH 0.5; pH 1; pH 2.5; pH 3.2) (AA), and modified Giemsa.

Histopathological diagnosis of the 77 cases of adenocarcinoma of upper gastric pole was performed according to OMS criteria [20] through the investigation performed been diagnosed 33 cases of tubular adenocarcinoma, 19 cases of papillary adenocarcinoma, 16 cases of non-differentiated adenocarcinoma, seven cases of “signet ring cell” adenocarcinoma and two cases of mucinous adenocarcinoma.

The immunohistochemical investigation has been performed only in 32 cases of adenocarcinoma of upper gastric pole, that corresponded on usual colorations to the following subtypes of gastric adenocarcinoma of the OMS: tubular (14 cases), papillary (nine cases), non-differentiated (five cases), with “signet ring cell” adenocarcinoma and two cases of mucinous adenocarcinoma.

As a control lot, we have used 20 cases of controls were obtained by removing from the above buffer–PBS, pH 7.4 at least two times. Negative incubations, the sections have been washed in phosphate incubation with normal 3% serum for 30 minutes. Then we have blocked the endogenous peroxidase reduction of the non specific connection through incubation with normal 3% serum for 30 minutes.

The incubation with the primary antibody was performed at 4°C over night using the antibodies mentioned in the table below.

Then we had minutes incubation with the biotinylated secondary antibody (anti-mouse or anti-rabbit considering the main antibody used), and then we went to the incubation with the complex peroxidase streptavidin (ABC<sup>kit®</sup>) for 1 hour.

The visualizing of the immunohistochemical reactions was performed with the help of diaminobenzidine (DAB), and the counter coloration was made with Hematoxylin Mayer. Between incubations, the sections have been washed in phosphate buffer–PBS, pH 7.4 at least two times. Negative controls were obtained by removing from the above technical the main antibody (Table 1).

The interpretation of the immunohistochemical relationships will firstly distinguish the chromogen at the level of pointed antigenic targets. The quantitative appreciation of the immuno-histochemical relationships between the used antibodies will be performed according to the score 1 below, the qualitative appreciation of the intensity of these colorations will be performed according to score 2 below and the grading of the expression of p53 and Ki67 is performed according to score 3 below (Table 2).

We mention that this research has been acquiescent by University Ethics Committee of University of Medicine and Pharmacy of Craiova, certificate no. 237/14.09.2006.
The immunohistochemical profile of the adenocarcinoma of upper gastric pole

Table 2 – Appreciation scores of the result of the performed immunohistochemical reactions

<table>
<thead>
<tr>
<th>Score 1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Positive cells</td>
<td>&lt;10%</td>
<td>10–25%</td>
<td>25–50%</td>
<td>50–75%</td>
<td>&gt;75%</td>
</tr>
<tr>
<td>Score 2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Intensity of the staining</td>
<td>weak</td>
<td>moderate</td>
<td>strong</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Score 3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Positive cells</td>
<td>negative</td>
<td>few nuclei</td>
<td>10%</td>
<td>10–50%</td>
<td>&gt;50%</td>
</tr>
</tbody>
</table>

Results

Thus, the histopathological investigation notify the predominance of the adenocarcinoma of tubular and papillary type (Figures 1a and 1b) having as localization the level of the gastric upper pole; histopathological types OMS who in the classification of Laurens correspond to the intestinal type of adenocarcinoma (representing 22.3% from the total of gastric adenocarcinoma of intestinal type).

The most frequently met cytological differentiation was that of a caliciform cell type (in 84% of the cases) (Figures 1c and 1d), followed then by: neoplastic cells seemingly from a morphologic point of view to those “in a brush” (51%), Paneth type cells (18.5%), neuroendocrine character for in 16.5% the neoplastic cells have been labeled as being non-differentiated because their morphologic characteristics did not correspond to any of the above presented cellular types.

In the study undertaken by us, we have noticed the prevalence of the fibrous type stroma (Figure 1e), stroma found in all of the undertaken cases. Reported to the stroma quantity we have noticed that the most frequently met type was the trabecular one with 53.2% in which we have noticed an equilibrated report between the quantity of tumoral parenchyma and stromal neoplastic elements.

These ones have been followed by gastric adenocarcinoma of a medullar type with a 35% percentage, schirous adenocarcinoma with a percentage of 11.8%, and in 30% of the cases, the stroma also had an inflammatory modified character, through the presence of a lymphoplasmocytic infiltrate.

The analysis of the casuistic taking into consideration the degree of cytological as well as cytoarchitectural differentiation proving the prevalence of the well differentiated forms with 53.25%, followed then by poor differentiated forms (Figure 1f) with 20.78%, moderate differentiated 18.18% and namely on the last place the non differentiated forms with 20.78%.

The immunohistochemical investigation with the antibody anti-Cytokeratin 7 (CK7)

From the quantitative point of view, the immunomarker anti-CK7 has predominantly presented a score 1 with 10–25% of the positive carcinomatous cells in 13 of the 32 investigated cases of adenocarcinoma of upper gastric pole (Figures 2b and 1c).

On the second place there was the quantitative score 2 with 25–50% of the positive tumoral cells met in 9 of the 32 cases (Figure 2a), maximal score-4 not being registered in any of the cases, and the minimal score with under 10% positive cells has been noticed in four cases (Table 3).

The qualitative analysis of the CK7 immunostaining according to the data registered in the above table has shown a predominantly qualitative of a moderate type score (score 2) present in 22 of the 32 investigated cases (Figure 2a).

The maximal qualitative score (score 3) has been notice in only six cases, while the minimal score (score 1, weak reaction) in four cases has been a cytoplasmic diffuse one (Figures 2b and 1c).

Table 3 – The qualitative and quantitative expression to the CK7 staining in the investigated cases of adenocarcinoma of upper gastric pole

<table>
<thead>
<tr>
<th>Score 1</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Positive cells)</td>
<td>(&lt;10%)</td>
<td>(10–25%)</td>
<td>(25–50%)</td>
<td>(50–75%)</td>
<td>(&gt;75%)</td>
</tr>
<tr>
<td>No. of cases / Total no.</td>
<td>4/32</td>
<td>13/32</td>
<td>9/32</td>
<td>6/32</td>
<td>0</td>
</tr>
<tr>
<td>Score 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(The intensity of staining)</td>
<td>weak</td>
<td>moderate</td>
<td>strong</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of cases / Total no.</td>
<td>4/32</td>
<td>22/32</td>
<td>6/32</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Reported to the histopathological variety of adenocarcinoma, the CK 7 immunostaining was more obvious to the tubular and papillary variant (Figure 2a) as compared to those in “signet ring cells” (Figure 2c) or mucinous.

The results of the investigation with the antibody anti-Cytokeratin 19 (CK19)

In what the quantitative CK19 immunostaining is concerned, according to the data presented in the table below, the predominant score was the one with 25–50% positive cells, namely score 2 present in 15 of the 32 cases of investigated adenocarcinoma of upper gastric pole (Figure 2d).

On the second place could be found the quantitative score 3 with under 50–75% of the positive tumoral cells met in nine of the 32 cases (Figures 2e and 2f).

The maximal score was presented in nine cases, and minimally in only one case (Table 4).

Table 4 – The qualitative and quantitative expression to the CK19 immunostaining in investigated cases of adenocarcinoma of upper gastric pole

<table>
<thead>
<tr>
<th>Score 1</th>
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<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Positive cells)</td>
<td>(&lt;10%)</td>
<td>(10–25%)</td>
<td>(25–50%)</td>
<td>(50–75%)</td>
<td>(&gt;75%)</td>
</tr>
<tr>
<td>No. of cases / Total no.</td>
<td>1/32</td>
<td>4/32</td>
<td>15/32</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Score 2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(The intensity of staining)</td>
<td>weak</td>
<td>moderate</td>
<td>strong</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of cases / Total no.</td>
<td>5/32</td>
<td>18/32</td>
<td>9</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Figure 1 – Adenocarcinoma of the upper pole: (a) tubular type, HE staining, ×40; (b) cystico-papillary type, HE staining, ×100; (c) cystico-papillary type – caliciform cells, Blue Alcian staining, pH 2.5, ×100; (d) caliciform cells, Blue Alcian staining, pH 2.5, ×400; (e) tubopapillary type with fibrous stroma, Goldner–Székely staining, ×100; (f) poorly differentiated adenocarcinoma, HE staining, ×100
Figure 2 – Adenocarcinoma of the upper pole: (a) tubular type, CK7+, QtS and QlS-2, ×100; (b) diffuse, poor differentiated type, CK7+, QtS and QlS-1, ×100; (c) signet ring cell type, CK7+, QtS and QlS-1, ×100; (d) tubopapillary type, CK19+, QtS-2 and QlS-3, ×200; (e) signet ring cell type, CK19+, QtS-3 and QlS-3, ×100; (f) mucinous type, CK19+, QtS-3 and QlS-3, ×40. QtS – quantitative score and QlS – qualitative score
The qualitative analysis of the immunomarker has shown the predominance of the moderate intensity of the reaction for CK19 in 18 of the 32 investigated cases. The maximal intensity of the immunostaining has been noticed in nine cases (Figures 2d, 2e and 2f), and the minimum qualitative score was presented in five cases. The pattern of the immunoreactions is a cytoplasmatic homogenous one.

Reported to the histopathological varieties we have presented a more intense immunostaining in the forms of an intestinal type with cells in "signet ring cell" (Figure 2e) and in the mucinous ones (Figure 2f) and weakly in the tubular (Figure 2d) and papillary ones. As a whole, the immunostaining has been a little bit more intense in the localization from the upper gastric pole as to the distal gastric localization of adenocarcinoma.

The immunohistochemical investigation with the antibody anti-Epithelial Membrane Antigen (EMA)

The immunostaining anti EMA from a quantitative point of view has predominantly presented a score 1 with 10–25% of the positive carcinomatous cells in 13 out of the 32 investigated cases of adenocarcinoma of upper gastric pole (Figures 3a, 3b and 3c).

On the second place there has been the quantitative score 2 with 25–50% of the positive tumoral cells met in 11 of the 32 cases, the maximal score 4 not being registered in any of the cases, and the minimal score with under 10% positive cells has been noted in four cases (Table 5).

Table 5 – The qualitative and quantitative expression of EMA antigen in the investigated cases of gastric adenocarcinoma of upper pole

<table>
<thead>
<tr>
<th>Score 1 (Positive cells)</th>
<th>0 (&lt;10%)</th>
<th>1 (10–25%)</th>
<th>2 (25–50%)</th>
<th>3 (50–75%)</th>
<th>4 (&gt;75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases / Total no.</td>
<td>4/32</td>
<td>13/32</td>
<td>11/32</td>
<td>4/32</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score 2 (The intensity of staining)</th>
<th>1 (weak)</th>
<th>2 (moderate)</th>
<th>3 (strong)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases / Total no.</td>
<td>8/32</td>
<td>22/32</td>
<td>2/32</td>
</tr>
</tbody>
</table>

From a qualitative point of view the EMA immunostaining as we can notice in the data from the table above, it has been a predominantly moderate one, fact noticed in 22 of the investigated cases. Maximum of intensity has been noticed in two cases and minimum in eight cases (Figure 3a, 3b and 3c).

The pattern of the immunoreaction to EMA has been an apical cytoplasmatically one, near the membrane. The immunostaining was more obvious in the well-differentiated forms with histological aspect predominantly tubular (Figure 3a) and tubulo-papillary, and a discrete immunostaining has been met in poorly differentiated forms, predominantly diffuse (Figure 3c).

An intermediary immunostaining has been noticed in the diffuse forms with "signet ring cells" (Figure 3b).

As compared to the EMA immunostaining from the investigated cases of carcinoma of the inferior gastric pole, in the proximal gastric localization, the immunostaining was inferior both as intensity as well as in quantity. We could not make correlations among the degree of expression of this antibody and the study of the disease.

The immunohistochemical investigation with the antibody anti-Carcinoembryonic Antigen (CEA)

The CEA immunostaining from a quantitative point of view has predominantly presented a score 2, the number of the positive carcinomatous cells being among 25–50% in 13 of the 32 investigated cases (Figure 3e).

According to the data written in the table below the qualitative analysis of the immunostaining at CEA was predominantly moderate, the qualitative score 2 being registered in 22 of the 32 investigated cases (Figures 3e and 3f).

The immunohistochemical pattern was one predominantly cytoplasmatic and rarely an apical cytoplasm one or in luminal secretion (Table 6).

Table 6 – The qualitative and quantitative expression of CEA in the investigated cases of gastric adenocarcinoma of upper pole

<table>
<thead>
<tr>
<th>Score 1 (Positive cells)</th>
<th>0 (&lt;10%)</th>
<th>1 (10–25%)</th>
<th>2 (25–50%)</th>
<th>3 (50–75%)</th>
<th>4 (&gt;75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases / Total no.</td>
<td>1/32</td>
<td>11/32</td>
<td>13/32</td>
<td>6/32</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score 2 (The intensity of staining)</th>
<th>1 (poor)</th>
<th>2 (moderate)</th>
<th>3 (strong)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases / Total no.</td>
<td>8/32</td>
<td>22/32</td>
<td>4/32</td>
</tr>
</tbody>
</table>

The most obvious immunostaining was for the poorly differentiated cases, at the contrary pole being the well-differentiated forms (Figure 3d).

The diffuse forms including those with "signet ring cells" (Figure 3e), mucinous (Figure 3f) and papillary have also presented an obvious immunostaining at the CEA marker.

As compared to the adenocarcinoma from the localization of the gastric inferior pole, the proximal ones have had an immunostaining less intense. Besides, we have noticed the presence of an intense staining also quantitatively as well as qualitatively in the advanced forms of gastric adenocarcinoma of upper pole, especially in the metastatic lymph nodes forms.

The immunohistochemical investigation with the antibody anti-Lysozyme

Quantitatively, the lysozyme immunostaining was a weak one, in the majority of the cases, namely 11 of the 32 investigated cases have presented a 0 score.

The maximal registered quantitative score was 3 that mean positivity under 75% of tumoral cells and those was obtained from the single case of mucinous adenocarcinoma.

All the other nine cases have presented the quantitative score 1 (Figure 4b), and other three the score 2 (Figure 4a) (Table 7).
The immunohistochemical profile of the adenocarcinoma of upper gastric pole

Figure 3 – Adenocarcinoma of the upper pole: (a) tubular type, EMA+, QtS and QlS-1, ×200; (b) signet ring cell type, EMA+, QtS and QlS-1, ×200; (c) diffuse, poor differentiated type, EMA+, QtS and QlS-1, ×200; (d) cystic-papillary type, CEA+, QtS-1 and QlS-1, ×40; (e) signet ring cell type, CEA+, QtS-2 and QlS-2, ×200; (f) mucinous type, CEA+, QtS-1 and QlS-2, ×40. QtS – quantitative score and QlS – qualitative score
Figure 4 – Adenocarcinoma of the upper pole: (a) tubulopapillary type, lysozyme+, QtS and QlS-2, ×100; (b) signet ring cell type, lysozyme+, QtS-1 and QlS-2, ×100; (c) diffuse, poor differentiated type, vimentin+, QtS-0 (<10% + cells) and QlS-2, ×200; (d) cystic-papillary type, VIM-, stroma is positive to VIM, ×40; (e) tubopapillary type, p53+, QtS-3 and QlS-3, ×200; (f) mucinous type, p53+, QtS-3 and QlS-3, ×40. QtS – quantitative score and QlS – qualitative score
From the qualitative point of view, the lysozyme immunostaining was a predominantly weak one, score 1 being met in of the 32 investigated cases. The maximal intensity of the reaction, namely score 3 has been present in only the case of mucinous adenocarcinoma. The pattern of the immunoreaction has been predominantly cytoplasmatic one in the tumoral cells, but the reaction has been also notice at the level of extracellular mucous. The reaction is comparable to that from the diffuse forms of gastric carcinoma with tubulopapillary predominantly pattern. A weak immunostaining has been noticed in the adenocarcinoma with “signet ring cells”, the pattern of the reaction being a peripheral cytoplasmatic one (Figure 4b).

The pure mucinous form is becoming positive at the level of lakes of mucus. The poorly differentiated type of gastric carcinoma of upper pole was negative to this immunostaining. Moreover, we have disposed a more obvious immunostaining in the advanced diseases with lymph nodes metastasis. As compared to the lysozyme immunostaining was more intense in upper localization.

The immunohistochemical investigation with the antibody anti-Vimentin

As a whole, the vimentin immunostaining has been a weak one both qualitatively and quantitatively. At the level of tumoral cells the immunostaining has been present in only three cases namely when there was about poorly differentiated adenocarcinoma (Figure 4c). The pattern of the reaction was a cytoplasmatic fibrilar one. In all the other cases the immunomarker was negative (Figure 4d). The rate of expression at vimentin was a little bit bigger in the distal gastric localization and this thanks to the great number of cases of adenocarcinoma poorly differentiated with this localization.

The immunoreactions were practiced especially for the immunohistochemical testing of the tumoral tissues, vimentin being used as a control. With a sporadic character focalized in stromal areas of the investigated cases we have pointed out a positive internal control at the level of cells with mesenchymal origin (fibroblasts, myofibroblasts, nervous ganglionic cells, smooth muscular fibers), as well as at the level of endothelial cells and of lymphocytes (Figure 4d).

The immunohistochemical investigation with the antibody anti-p53

As a whole the quantitative expression of the investigated cases is heterogeneously distributed between score 0 (with less than 10% of the tumoral positive cells) and score 4 (in no case). As we can observe from the data presented in the table below we could not place any of the investigated cases in score 4 quantitative, the biggest part of the investigated cases of adenocarcinoma of upper gastric pole quantitative score 3, namely 12 out of the 32 cases (Figures 4e and 4f) (Table 8).

<table>
<thead>
<tr>
<th>Score 1</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cells (%)</td>
<td>(&lt;10%)</td>
<td>(10–25%)</td>
<td>(25–50%)</td>
<td>(50–75%)</td>
<td>(&gt;75%)</td>
</tr>
<tr>
<td>No. of cases / Total no.</td>
<td>11/32</td>
<td>9/32</td>
<td>3/32</td>
<td>1/32</td>
<td>0</td>
</tr>
</tbody>
</table>

The pattern of the reaction was a predominantly nuclear one, rarely noting a cytoplasmatic perinuclear pattern. From the point of view of the intensity of reaction, as it can be noticed from the data presented in the table below the biggest part of the investigated cases have had a reaction of moderate intensity (score 2 of intensity), namely 23 of the 32 cases of adenocarcinoma of upper gastric pole. Only in two cases we have noticed a maximal score 3 of the intensity of immunoreactions at p53 (Figures 4e and 4f).

Referring to the histological type we have registered a more intense immunostaining in the intestinal part than in the diffuse one (Figure 4f), and in what tumoral grading is concerned the well differentiated forms (Figure 4e) the immunostaining for p53 has been superior to the poorly differentiated forms.

As a plus we have noted an immunostaining at p53 more obvious in the advanced cases of adenocarcinoma and especially in those with lymph nodes metastasis.

Comparatively looking at the results of the immunoreexpression of p53 at the level of the upper gastric pole to the distal localization of the cases of gastric carcinoma we have noted both quantitatively and qualitatively a superior immunostaining in the proximal localization.

The immunohistochemical investigation with the antibody anti-Ki67

The Ki67 immunostaining has been noticed in all of the investigated cases and was an exclusively nuclear one. According to the data presented in the table below (Table 9) the biggest majority of the cases have presented a moderate immunostaining, namely 20 of the 32 investigated cases of adenocarcinoma of upper gastric pole (Figure 5c).

The intensity of the immunostaining was maximal especially in the cases with diffuse pattern and poorly differentiated (Figure 5a). The cases with the weakest immunostaining have been represented by two of the cases of adenocarcinoma in “signet ring cells” (Figure 5b) and the only case of mucinous adenocarcinoma.
Figure 5 – Adenocarcinoma of the upper pole: (a) diffuse, poor differentiated type, Ki67+, QtS-4 (>50 + cells) and QlS-3, ×200; (b) signet ring cell type, Ki67+, QtS-1 (few nucleus) and QlS-1, ×100; (c) tubopapillary type, Ki67+, QtS-4 (<50 + cells) and QlS-2, ×100; (d) diffuse, poor differentiated type, CD34+, high MVD (44.9 ± 18.7) and QlS-3, ×100; (e) mucinous type, CD34+, low MVD (15 ± 10.2) and QlS-1, ×200; (f) tubulopapillary type, CD34+, moderate MVD (25.6 ± 13.5) and QlS-2, ×100. QtS – quantitative score and QlS – qualitative score.
As to the quantitative appreciation of the reaction for Ki-67 we have noted that the biggest majority of the studied cases obtained score 3, namely 17 of the 32 investigated cases. In poor differentiated cases and those with diffuse pattern the number of positive nuclei was over 50% (Figure 5a), and in single mucinous case the positive nuclei number was lower than 10% (Figure 5b).

Table 9 – The qualitative and quantitative expression of Ki67 in the investigated cases of gastric adenocarcinoma of upper pole

<table>
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<tr>
<th>Score 1</th>
<th>(The intensity of staining)</th>
<th>1 – poor</th>
<th>2 – moderate</th>
<th>3 – strong</th>
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<table>
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<tr>
<th>Score 2</th>
<th>(Positive cells) negative (few nuclei)</th>
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<th>2</th>
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<td>(Positive cells) negative (few nuclei)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

As compared to the cases of adenocarcinoma of inferior gastric pole investigated for the same marker there are no significant differences. The difference should stay in the obtaining of a higher score in what the number of positive nuclei is concerned (from the 20 investigated cases 14 had score 4) and this because of the biggest number of diffuse cases having this localization.

Referring to the Ki-67 proliferate index the medium obtained value for the cases of adenocarcinoma of upper gastric pole was of $43 \pm 16.8$ little under the one obtained for the one of inferior gastric pole $49 \pm 19.3$.

We have also noted a biggest value of this index as reported to the degree of differentiation, the biggest values being registered in the poorly differentiated forms ($62 \pm 23.6$ vs. $23 \pm 11.6$ in the well differentiated forms) and with the study of the disease, maximal values being registered in the advanced stages. A minimal difference has also been noticed as reported to the histological type, thus, the diffuse forms have presented values a little bit bigger than those of an intestinal type have. This was more obvious especially in the gastric distal localization.

The immunohistochemical investigation with the antibody anti-CD34

The CD34 immunostaining has been present in all of the investigated cases, but, in exchange, the intensity of the immunostaining was predominantly moderate (score 2 of intensity) (Figure 5f) met in 17 out of the 32 immunohistochemical investigated cases (Table 10).

Table 10 – The qualitative and quantitative expression of CD34 in the investigated cases of gastric adenocarcinoma of upper pole

<table>
<thead>
<tr>
<th>Score 1</th>
<th>(Positive cells) (&lt;10%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td></td>
<td>(Positive cells) (&lt;10%)</td>
<td>0</td>
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<table>
<thead>
<tr>
<th>Score 2</th>
<th>(The intensity of staining)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>–</th>
<th>–</th>
</tr>
</thead>
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<tr>
<td></td>
<td>(The intensity of staining)</td>
<td>1</td>
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<td>3</td>
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Score 3 of intensity has been noticed in 12 cases (Figure 5d), and score 1 in only three cases (Figure 5e). Maximum of intensity has been noticed in the poorly differentiated forms (Figure 5d), and the minimum was observed in the mucinous forms (one case) (Figure 5e) and two of the three cases of adenocarcinoma with “signet ring cells”. Moderate intensity is characteristic to the moderate differentiated forms of a tubular, papillary and tubulo-papillary type (Figure 5f).

The pattern of the immunostaining has been a membrane one, delimiting sometimes well visible lumina, and some other times the immunostaining was limited to endothelial isolated cells.

The degree of maturity of the vessels is variable, form new vessels that appear as single isolated endothelial cells to vessels with visible lumina, and sometimes with aspects of budding. As compared to the cases of adenocarcinoma of inferior gastric pole, the intensity of the immunostaining was a small one, the majority of the cases of pyloric carcinomas being of a diffuse type.

The appreciation of vascular microdensity led to the obtaining of a value of $32.4 \pm 13.4$ per microscopic field on a 200 magnification. Referring to the histologic type, bigger values of up to $45.3 \pm 19.3$ was obtained in the poorly differentiated forms (Figure 5d), while small values $14 \pm 9.2$ in the well differentiated forms (Figure 5f) and in the single case of mucinous adenocarcinoma (Figure 5e).

Reported to the cases of gastric adenocarcinoma of inferior pole the values of vascular microdensity are a little bit smaller, the medium obtained value in these cases being of $41 \pm 16.3$. In addition, here also, we have noticed the presence of big values in the poorly differentiated and mucinous forms (better represented from a numeric point of view); while the well differentiated forms of a tubulo-papillary type have had smaller values than the average.

Another observed fact is that vascular microdensity was bigger in the cases with lymph nodes metastasis.

Discussions

The histopathological investigation according to the data in the specialty literature pointed out:

- The predominance of a diffuse form according to Lauren’s classification and respectively tubulo-papillary according to OMS classification in the proximal localization towards the distal one with a tendency to increase the incidence for the intestinal type.
- The predominance of the well differentiated forms of adenocarcinoma at the level of the gastric upper pole, but closely followed by the non-differentiated forms.
- The clinical diagnosis in made in far more advanced stages (IIIB and IV) of cardiotuberozitar carcinoma and of the gastric bottom than the antral ones.
- The biggest tendency to profound invasion of proximal carcinoma towards those with antral localization, as well as an increased incidence of lymphatic dissemination of the adenocarcinoma of gastric upper pole as compared to those located in the distal gastric zone.
Anti-CK7 and anti-CK19 immunostaining

Our analysis has pointed out the fact that the biggest part of the adenocarcinoma of gastric upper pole (41%) have had a quantitative score 1 in the CK7, while the quantitative immunostaining in CK19 has shown a greater number of cases with score 2, respectively 47. From a qualitative point of view the immunostaining of the adenocarcinoma of gastric upper pole was predominantly moderate with 69% of the cases, while in what the qualitative immunostaining in CK19 is concerned we have noticed the predominance of score 2 – moderate intensity, namely 56% of the investigated cases.

Histopathologically the immunoreaction to CK7 was clearly visible in the tubular and papillary variants as compared to those in “signet ring cell” or mucinous. In the case of the immunostaining at CK19 the reaction has been more intense in the forms of intestinal type with “signet ring cells”, and in the mucinous ones and weaker in the tubular and papillary ones. As a whole the immunomarker at CK7 has been weaker in cardia and fundic localization than in the body and gastric- antral one, while for the immunomarker at CK19 the reaction has been more intense in the localization at the level of gastric upper pole towards the distal gastric localization of the adenocarcinoma.

Cytokeratins constitute a group of intermediary filaments present in normal epithelium and in the tumors derived from them [21]. However, a special practical interest is presented by the expression of cytokeratin CK7 and CK 20 in different tissues [21, 22]. CK20 is a marker of the intestinal differentiation at surface and in the cryptic epithelium of the intestine, while CK7 is present in the tissues having the origin in the intestinal metaplasia of the esophagus.

A series of immunohistochemical studies have tried to establish a cytokeratin profile capable to differentiate Barrett metaplasia, from the intestinal one of the stomach. That it was proved a powerful superficial immunostaining at CK 20 and namely an intense reaction to CK7 both of the superficial glands and of the profound ones from Barrett esophagus, while such intestinal cardia metaplasia an immunohistochemical profile was absent at the level of cardia and subcardial intestinal metaplasia [23].

Fluke U et al. [24], have note dated at the level of intestinal metaplasia a powerful CK7 reaction both in superficial glands as in the profound ones, as well as a limited CK20+ reaction only in superficial glands. This cytokeratinic profile has been also noted by Glickman JN et al. [25]. However, other authors [25] did not note such differences in what CK7/20 immunoreactivity between the short and long segments of the Barrett esophagus and the intestinal metaplasia of the eso-gastric junction.

Ormsby AH et al. [26] have noticed that the phenotype CK7+/CK20- is sensible and specific for esophageal carcinoma developed on Barrett esophagus. This thing has been ulcerate confirmed by Taniere P et al. [27], the authors suggesting that the profile of the CK7/CK20 expression can be useful in the differentiation of the adenocarcinoma of eso-gastric junctions. Thus, by investigating a number of 85 adenocarcinoma of distal esophagus and 67 adenocarcinoma of proximal stomach have noted the fact that 90% of the distal esophageal adenocarcinoma have been positive for CK7 and CK19, as compared to 45% of the adenocarcinoma of proximal stomach; 17.6% of the adenocarcinoma of distal esophagus and 55.2% of those of proximal stomach have expressed CK20 and 74.1% of the adenocarcinoma of distal esophagus and 23.8% of the adenocarcinoma of proximal stomach have had an CK7+/CK20-immunophenotype. For the intestinal subtype of adenocarcinoma the CK7+/CK20- immunoprofile had a sensibility of 76.5%, a specificity of 84.5% and a predictive value for the diagnosis of adenocarcinoma of distal esophagus of 87.3%.

Unlike these authors Gulmann C et al. [28], investigating immunohistochemical 14 esophageal carcinomas, 39 carcinomas of the eso-gastric junction and 78 gastric carcinomas (both of the antrum and body) have noted the fact that Barrett cytokeratinic phenotype (CK7+/CK20-) has been present in 7% of the esophageal carcinoma, 18% of the eso-gastric and 13 of those exclusively gastric ones. This kind of values indicates the fact that the phenotype CK7+/CK20- would be useful in the differentiation of esophageal carcinoma from the rest of gastric carcinoma. The phenotype CK7+/CK20+ has been expressed in 14% of the esophageal carcinoma, 3% of the eso-gastric carcinoma and 1% of the gastric carcinoma, while the immunophenotype CK7+/CK20+ has not been identified in any of the investigated cases, and the phenotype CK7-/CK20- has been present in 79% of the investigated esophageal adenocarcinoma, 79% of the eso-gastric and 86% of the gastric ones [30].

Fluke U et al. [24] have proved that there are no significant differences concerning the cytokeratinic pattern at the level of the adenocarcinoma of distal esophagus, eso-gastric junction and of the proximal stomach. Besides, the presence or absence of Barrett’s metaplasia does not influence the expression of cytokeratins. As opposed to Ormsby’s AH et al.’ results [26] that were identifying a phenotype CK7+/CK20-characteristic to esophageal adenocarcinoma with Barrett metaplasia. Flucke U et al. [24] did not note such cytokeratinic profile for these tumors and in exchange have proved that all these types of tumors have been CK20+. The frequency of the CK20+ adenocarcinoma signaled by Fluke U et al. [24], have been totally superior that the one signaled by Sarbia M et al. [29].

These differences, especially those concerning the expression CK20 can be explained by: 1) the selection of cases (small number of cases or the unseselecting of rigorous topographic criteria of harvesting specimens); 2) the usage of different monoclonal antibodies with variable epitopes specifications; 3) the usage of methods and kits of different immunohistochemical colorations; 4) the usage of different scores of quantitative and qualitative appreciation of the immunohistochemical reactions.
Recently, Mattioli S et al. [30], have investigated immunohistochemically 62 esophageal adenocarcinoma (15 cases), cardial (37 cases) and antral gastric (10 cases). The authors have pointed out the existence of a cytokeratonic pattern of a Barrett type (CK7+/CK20-) in 35% of the cardial adenocarcinoma, and the rest 10 of 37 with a pattern type CK7-/CK20+ and namely 14 of 37 with CK7+/CK20+ profile. Esophageal adenocarcinoma has presented a Barrett profile in 87.5% both in the tumor as well as in the zone with intestinal metaplasia. The adenocarcinoma of the gastric antrum in a percentage of 100% did not present the Barrett phenotyope.

Therefore, it seems not to be present a predominant pattern CK7/CK20 of gastric adenocarcinoma, a significant percentage of these becoming positive for each of the possible pattern CK7/CK20. About 35% of the gastric adenocarcinoma are CK7+/CK20+, 25% are CK7-/CK20+, 25% CK7+/CK20- and 15% CK7-/CK20- [22, 31–38]. These percentages varying up to 30%.

Reported to the histopathological variety of adenocarcinoma CK7, different authors have noted the more intense expression of CK7 in the tubular and papillary variants as compared to those in “signet ring cells” or mucinous [24].

The immunostaining at the antibody anti-EMA

The quantitative EMA immunostaining has been a predominant one (41%) with score 1 (with 10–25% positive cells), and from a qualitative point of view the immunostaining has been a predominantly moderate one, met in 69% of the investigated cases.

The maximum of reaction’s intensity at EMA has been noted in the well-differentiated cases with increasing tumoral pattern predominantly tubular and tubulo-papillary, and the minimum has been noted in the poorly differentiated and diffuse forms of adenocarcinoma.

As a whole, the EMA immunostaining of the cases of adenocarcinoma of upper gastric pole has been inferior to the cases with distal gastric localization. We also mention that we could not establish correlations between the degree of EMA expression and the stages of the disease.

Pinkus GS and Kurtin PJ [39] have investigated the immunohistochemical expression of EMA in 320 adenocarcinoma with primary origin in the mammary gland, lung, stomach, urinary bladder, prostate, endocrine glands, ovary, kidneys and thyroid, finding a percentage of positivity of the immunoreactions in 91% of the cases, and the most frequently met pattern has been an apical cytoplasmatic membrane one. The obtained results by these authors have proved that EMA is an excellent epithelial differentiation marker, seeming to discriminate very well the poorly differentiated carcinoma of malignant lymphoma and in the same time; it is useful in the characterization of anaplastic carcinoma with small cells [41].

Thomas P and Battifora H [40] have comparatively studied the sensibility and specificity of cytokeratin immunomarkers in neoplastic epithelial proliferations (AE1, CAM 5.2, PKK1, and 35 beta H119) versus EMA.

The authors have found that the anti-EMA immunostaining has been less sensitive than the cytokeratonic one in different epithelial tumors, including gastrointestinal carcinoma (13 of 34 being negative).

Moreover, authors have noted results falsely positive to the immunoreaction with anti-EMA in two of the 14 cases of lymphoma with T-lymphocytes. As a conclusion, the monoclonal antibodies directed against cytokeratins with small molecular weight are far more sensitive and specific in the identification of epithelial origin of neoplasia than the monoclonal anti-EMA antibody [40].

Monig SP et al. [41] have shown the utility of using immunostaining with antibody anti-EMA in the rapid intrasurgical evaluation of the safety oncology borders and the quick intrasurgical diagnosis of lymph nodes micro metastases in gastric carcinoma.

Wang GY et al. [44] have proved the utility of using immunohistochemical investigation together with antibodies anti-CK20, anti-EMA and anti-CEA in the discovering of lymph nodes micro metastases in patients with gastric adenocarcinoma.

The Carcinoembryonic Antigen (CEA) immunostaining

The biggest part of the investigated cases about 41% have presented a quantitative score of CEA immunostaining of type 2 with 25–50% of the positive carcinomatous cells, and from a qualitative point of view the majority of the cases, about 69% have had a score 2- of medium intensity.

From the point of view of the tumoral grading, the poorly differentiated forms have presented the most obvious immunostaining from the point of view of the diffuse increasing pattern it has been the most sensitive to this immunostaining. Histologically, the intestinal type has presented an immunostaining superior to the purely gastric one. As a whole, the CEA immunostaining of the cases of adenocarcinoma of upper gastric pole has been inferior to the cases with distal gastric localization. Moreover, we have noted an intense immunostaining in the advanced stages of the disease, especially in the forms with lymph nodes dissemination.

Sheahan K et al. [43], have investigated the reactivity of the carcinoembryonic antigen and of the mono and polyclonal antibodies anti-CEA in different malignant epithelial neoplasia. The authors have noticed a constantly positive immunoreactivity to all five antibodies anti-CEA investigated in the gastrointestinal adenocarcinoma. The conclusion of this study was that the immunoreaction at CEA might help at the identification of the histogenesis of the epithelial tumors [43].

Nielsen K and Teglbjaerg PS [46] have studied immunoreactivity at CEA of 92 cases of gastric adenocarcinoma and have identified the existence of three types of immunohistochemical phenotypes:
1. Intracytoplasmatic granules scattered in the entire cytoplasm of malignant cells.

2. Linear or granular deposits along the glicocalix or in apical cytoplasm of malignant cells.

3. Tumoral malignant cells with a weak reaction limited only to a few cells.

Reporting these phenotypes to the morphologic variants of gastric adenocarcinoma was observed that type I of pattern CEA is present in the majority of cells from the mucinous variant of adenocarcinoma, type II to the intestinal variant of adenocarcinoma, while type III of pattern CEA was present in pyloro-cardiac carcinoma.

Berner A et al. [45], have tested the immunoreactivity of three monoclonal anti-CEA antibodies CEA (12–140–1, –2 and –4) in gastric lesions and have noticed the positivity in 13, 11 and 13 of the 14 diffuse gastric carcinoma, in 11, 13 and 13 of the 18 tested intestinal carcinoma and the positivity in 9, 9 and 6 of the 34 studied dysplastic lesions. All of the tested monoclonal antibodies have presented the same type of pattern and did not have crossed reactions. Monoclonal antibodies unlike polyclonal ones color more cells, their reactivity being limited to intracytoplasmatic vacuoles in the diffuse types of carcinoma and one diffuse cytoplasmatic or limited to the cellular membrane in the adenocarcinoma of intestinal type. The obtained results determined the authors to say that the CEA antigen does not represent a specific marker of malignant transformation in gastric mucous [45].

Wu JF [48] has studied immunohistochemically the relationship among the CEA expression and the histogenesis of gastric cancer in 134 cases. The authors have noted a positivity of the immunoreactions in 85% of the analyzed cases, including the mucinous, carcinoma with “signet ring cells”, and papillitary carcinoma variants.

In the variant of tubular adenocarcinoma was noticed a tendency of increasing the CEA expression with the degree of cellular differentiation. The rate of CEA expression was bigger in gastric carcinoma producing sulphomucines as compared to those missing this kind of mucins. These results come to certify the histogenesis of gastric cancers positive CEA positive in the intestinal metaplasia of colonial type, while negative carcinoma at CEA seems to develop in the native gastric epithelium [46].

Ychou M et al. [47] have followed clinical signification and the value of the marker CA72-4 compared to CEA and CA19-9 to the patients with gastric ulcer. The sensibility of these tumoral markers in the neoplastic lesions from the level of tumoral margins was of about 58% and has increased to 75% for the patients without metastasis. Patients with abnormal values of CA72-4 seem to have a rate of survival smaller than those with normal values. In the patients with metastasis only the increased values of CA19-9 seem to be indicators of prognostic in the invariant analysis, while the multivariate analysis have shown the fact that both CA72-4 as well as CA19-9 have been independent factors of diagnosis. As a conclusion, this study suggests the fact that by adding CA 72-4 to CEA and/or CA 19-9 may help the well-being of the sensibility of the immunomarker in gastric cancer [47].

Takahashi Y et al. [48] have investigated the utility of CEA and/or CA 19-9 in the monitoring of recurrences in gastric cancer. Investigating 321 cases, the authors have noticed a sensibility of 65.8% for CEA and CA19-9 and namely 85% for both markers in tumoral recurrences, values a little bigger than the post surgical ones (28.3% and 45%). The results suggest the fact that these markers may be useful in the post surgical monitoring of the patients with gastric cancer, having a prognostic value for tumoral recurrences especially in the cases that have presented post surgically bigger values of these markers [48].

**The Lysozyme immunostaining**

As a whole, the immunostaining at lysozyme has been a weak one, over 65% of the cases having qualitative scores 1, and quantitative a majority, that is 34.37% have presented under 10% cancerous cells positive at lysozyme. Maximum of intensity has been noticed in the case of mucinous adenocarcinoma, both in the cytoplasm of carcinomatous cells from glandular structures as well as in mucous lakes and peripherical cytoplasm of the “signet ring cells”.

As compared to distal immunostaining has been more obvious in the localization from the level of upper gastric pole of the adenocarcinoma. In addition, the immunostaining at lysozyme seems to correlate also to the stadium of the disease because we have noticed an intense immunostaining in the advanced forms of the disease and especially in those with lymph nodes metastasizing.

Santini D et al. [49] have studied from an electro microscopic and immunohistochemical point of view the distribution of the lysozyme in normal and pathologic gastric and intestinal mucous. Thus, the authors have found that lysozyme is present in the epithelial cells of pylori glands, Paneth cells and some cryptic cells of colic mucosa.

As a plus, the lysozyme has been also detected in every large number of “immature” and “regenerative” epithelia: the colet cells from the level of regenerative gastric zones, columnar cells non differentiated by the surface and those from the level of hyperplasic crests of the stomach, regenerative cells from gastric ulcer that is about to be healed, some caliciform cells from incomplete intestinal metaplasia, the cells of the regenerative zones from the foetal intestinal epithelium.

Electron microscopically the lysozyme has been located in the center of mucous grains of the epithelium of gastric pylori glands and in the dense mucous granules from mucous cells of the colet of gastric pyloric glands. The lysozyme has been also detected in some of the cells producing immature mucous of gastric regenerative zones, as well as in the rough endoplasmic reticulum of gastric columnar cells of hyperplasiated surface. All these results led to the idea that the presence of lysozyme can be tied to the immaturity of the cells as well as to their regenerative strata [49].
The distribution of the lysozyme and its relationship to mucosubstances in gastric and colon carcinoma suggest the idea that lysozyme does not have to be considered an exclusive marker of the engagement of neoplastic cells as differentiated from the gastric type.

Xie ZX [50] has performed an immunohistochemical and ultrastructural study on the presence of endocrine neoplastic cells and of Paneth cells in gastric carcinoma. The author has pointed out the presence of lysozyme secretion in 89 from the 128 investigated gastric carcinoma, and in 69 cases the tumors have presented simultaneously in different cells more than two hormones or only one hormone and lysozyme. Reported to the histologic subtype of gastric carcinoma the author has noted a rate of expression of the lysozyme both isolated and in association with the fact that endocrine neoplastic cells and Paneth cells for neuroendocrine hormones. The results indicate was pointed out a bigger rate of lymph nodes metastases than in those of an intestinal type. Moreover, there was pointed out a bigger rate of lymph nodes metastases for positive gastric carcinoma for the lysozyme and for neuroendocrine hormones. The results indicate the fact that endocrine neoplastic cells and Paneth cells that have a common origin in the stem gastric neoplastic cells with pluridirectional potential of differentiation [50].

Yi YF and Huang YR [51], investigating the production of hydrolases in cancerous cells from different types of gastric carcinoma have noticed an immunoreaction for great intensity lysozyme in mucinous carcinoma, this varying according to the type of cytological differentiation. In the well-differentiated adenocarcinoma, the reaction has prevailed at the level of apical cytoplasm of glandular structures, while in the poorly differentiated forms the majority of cancerous cells have been negative. The subtype with cells in ring with seal has presented a weaker immunoreaction limited only to peripheral cytoplasm. Moreover, the authors have noted a positive reaction also to the level of mucus lakes suggesting the participation of this enzyme to the processes of degradation of the proteoglycan from extra cellular matrix’s constitution, thinking that could ease the invasion and metastasis in these forms of gastric cancer [51].

The Vimentin immunostaining

The immunohistochemical investigation of the cases of adenocarcinoma of upper gastric pole at vimentin was present only in three of the five cases of poorly differentiated adenocarcinoma that represented less than 9.35%. The percentage of positive case sat this marker has been bigger in gastric distal localization, namely 13% and this because of the great number of cases of poorly differentiated gastric adenocarcinoma developed in this location. In the rest of the investigated cases, the marker was positive with focal character in the cytoplasm of the mesenchymal type cells: fibroblasts, myofibroblasts, adipocytes, smooth muscular fibers, ganglionary nervous cells and besides, at the level of lymphocytes and of the endothelial cells.

The immunohistochemical staining for vimentin finds its utility in the case of gastric adenocarcinoma only in the poorly differentiated forms or in those with differentiations of the rhabdoid and sarcomatoid type.

In 1996, Utsunomiya T et al. [52], investigating immunohistochemically 239 gastric adenocarcinoma poorly differentiated with predominantly solid pattern have noted an expression of vimentin in 6.3% of the cases. In eight of the investigated cases was noticed a simultaneous diffuse co expression of vimentin and cytokeratin CAM 5.2; these cases being made out of a diffuse proliferation of polygonal cells, that on certain areas have presented connected intracellular contacts. The prognostic of these was worse than of the vimentin-negative ones [52].

In 2002, Utsunomiya T et al. [53] have studied with help gastric vimentin-positive carcinoma. The cytokeratinic staining was one of a membranous type, localized predominantly just under the cellular membrane both in the solid and in the tubular areas, but in the diffuse ones the membranous staining was usually absent. The vimentin immunostaining pattern in all proliferative areas was a fibrilar one, but in those with tubular cytoarchitecture the immunostaining was specifically basal one. This study comes to prove the fact that the presence of vimentin in gastric adenocarcinoma could not be due to the simple replacing of weight by vimentin, taking into consideration the different distribution of the two antibodies in the cytoplasm of tumoral cells [53].

Pinto JA et al. [54] have described a case of gastric adenocarcinoma well differentiated with areas of rhabdoid differentiation positive immunohistochemical for vimentin, but negative for cytokeratins and for the epithelial membrane antigen (EMA). The authors have suggested that this tumoral type would be a phenotypic distinctive variety of gastric adenocarcinoma [54].

Ueyama T et al. [57] investigating 71 gastric adenocarcinomas of a solid type have noticed that some case were vimentin positive. In all of these cases was noted an alveolar or diffuse pattern, tumoral cells being poorly cohesive or completely discohesive. Moreover, many of the tumoral cells have a rhabdoid malignant phenotype present and have coexpressed vimentin and cytokeratin.

Rivera-Hueto F et al. [56] have reported a case of precocious gastric adenocarcinoma developed on the gastric stump who histologically speaking has presented areas with rhabdoid type differentiations. These areas have presented vimentin and/or cytokeratins at a double immunostaining [56].

Carcinosarcomas are biphasic tumors whose incidence at the level of the stomach is very reduced [57–64]. Histologically, this kind of tumor is composed out of epithelial and mesenchymal components. Very often though there are many cellular lines different phenotypically, thing that suggests the origin of these tumors in stem cells with pluridirectional potential of differentiation pluridirectional. When tumour cells with fusiform morphology express vimentin, desmin and actin is about a differentiation of muscular type of the sarcomatous component.

Watanabe M et al. [65], have shown immunochemically through the immunostaining at
vimentin and act in the presence of myofibroblasts in the stroma of the gastric adenocarcinoma, much more numerous in the poorly differentiated forms and largely invasive. Moreover, the authors have noted a bigger number of myofibroblasts at the periphery of tumors than in their middle [65].

**The p53 immunostaining**

The maximal score of quantitative expression of p53 number of the n investigated cases of adenocarcinoma of upper gastric pole has been registered in 37.5% of the cases was a score 3, that is the number of positive tumoral cells in this marker was below 75%. In what the intensity of the immunostaining is concerned the majority of cases (71.87%) have presented a score 2, that means they had a qualitative moderate reaction. The most obvious immunostaining at p53 has been noted in the cases of adenocarcinoma of intestinal type, as well as in the well-differentiated forms. At the opposite pole, the cases of adenocarcinoma of upper gastric pole of a diffuse type and poorly have been differentiated.

Concerning comparatively the rate of expression of p53 in the adenocarcinoma of upper gastric pole, we have noticed the obtaining of higher scores qualitatively and quantitatively in the proximal localization towards the distal one.

Shun CT et al. [66] have investigated immunohistochemical the expression of p53 and c-erbB-2 in 112 cases gastric of adenocarcinoma. The authors have noted a positive immunostaining in 61 out of the 112 cases investigated, representing a percentage of 54.5%. There was found no correlation between the expression of p53 and sex, age, lymph nodes metastases, the infection with H. pylori or the prognostic of the disease. However, it was observed a significantly increased expression of p53 in the advanced forms of gastric carcinoma (60.7%), the intestinal variant (69.8%) and cardiac region (76.5%) towards the precocious ones (30.4%), the diffuse variant (34.7%) and non-cardiac regions (50.5%) [66].

Ichiyoshi Y et al. [67] investigating the immunohistochemical expression of p53 in 196 de cases with gastric cancers in advanced stages they noted a rate of positivity of about 48%, not correlated to the stage of the disease or the profundness of the invasion, but correlated to the rate of vascular invasion and the degree of lymph nodes metastasizing. In columnar variants and with vertical increase, namely the rate of positivity was of 53.8%. In the type characterized by increasing and dissemination the rate of expression of p53 was significantly lower 28.9%. The rate of survival at five years was of 44.2% and 25.4% for the patients with negative p53 and namely with positive p53. These results have shown that the altering of p53 is associated to a bad prognostic in the advanced studies of gastric cancer, allowing the tumor to increase in the depth of the gastric wall [67].

Honda T et al. [68] have investigated the expression of tumoral suppressors and of some of the proteins expressed by the tumor, including glycoprotein CEA in the different types of gastric adenocarcinoma. The authors have noted that the type of adenocarcinoma purely undifferentiated has presented the lowest rate of expression of p53, p16, hMLH1 and c-erbB-2, but the highest for CEA. The expression of p53 has been the most intense in the differentiated type of gastric adenocarcinoma (26.6%) and the lowest in the purely undifferentiated (15.2%). Moreover, it has already been noted over the expression of p53 more than the precocious tumoral stages (Tis and T1) than in the advanced ones (T2–T4). It has been seen a decreased rate of expression of p53 to young patients and in lymph nodes metastasis [68].

The existence of similar alterations of oncoprotein p53 in the squamous esophageal carcinoma and gastric cardial adenocarcinomas from the same patient indicates the possibility of implication of two similar cellular mechanisms that offers important molecular and etiologic arguments concerning similar geographic distribution as well as that of the risk factors in the two distinct tumour entities [69–70]. These results suggest that some of p53 mutations may have a tumorigen selective advantage during tumoral progression.

Moreover, Li HL et al. [71], have suggested that p53, bcl-2 and caspase-3 may play a very important role in the induction of apoptosis in the cells of cellular line AGS of human gastric adenocarcinoma. During the formation of gastric carcinoma, the proliferation of the gastric mucous may be intensified by the infection with H. pylori, intensifying consecutively and the expression of p53 mutagenic gene [72].

Gleeson CM et al. [73] have compared the mutations of p53 in the adenocarcinomas of distal esophagus and that of cardia and have reported a frequency of 70% of the mutations in esophageal adenocarcinomas and 63% in cardial adenocarcinomas. 85% of the mutations of p53 in the esophageal adenocarcinomas have presented the transactional type G:C A:T with 69% at the level of dinucleotides CpG. Similar mutations have been noted also in the cardial adenocarcinoma, in which 82% have been basic transactions out of which 55% have had a place at the level of dinucleotides CpG. In a study performed on Chinese population from the region Linzhou (population presenting the highest risk of esophageal and cardial carcinoma of the world) in which were investigated the mutations of p53 have been identified six mutations in 14 gastric cardial adenocarcinoma, three being G-T transversions, a mutation rarely observed in the gastric adenocarcinoma [74].

Flejou JF et al. [75] have performed a comparative study concerning the expression of p53 in o esophageal and gastric adenocarcinoma and have reported an overexpression a lot bigger of this oncoprotein in cardial gastric and esophageal carcinoma towards the antral one. This thing has been confirmed by Ireland AP et al. [76], the authors observing a significantly smaller rate of expresressing of p53 in gastric distal adenocarcinoma as compared to the esophageal and cardial ones. Moreover, a higher incidence of the expression was found among young patients, these having a worse diagnosis. The results are similar to those reported by Strickler JG et al. [77], who have sustained the existence of
mutations p53 in six of the 14 cases (43%) of adenocarcinoma developed at the level of eso-gastric junction as compared to only 2 cases out of the 26 (28%) investigated gastric distal adenocarcinoma. All these results indicate on the one side the existence of a common physiopathological way of cardia and esophageal tumors, on the other side the fact that the gastric cardia adenocarcinoma are a completely different entity from gastric distal adenocarcinoma.

The Ki67 immunostaining

In the majority of the investigated cases, namely 62.5% have presented a moderate intensity at Ki67 immunostaining, in what the quantitative staining for Ki67 is concerned for their majority, that is 53.12% have presented score 3. Reported to the histological type and grading we have noted both quantitatively as well as qualitatively positive reactions for the marker Ki67 more obvious in their diffuse forms than in the intestinal ones and namely in the poorly differentiated as faced to the well differentiated ones.

Porschen R et al. [78] have investigated with the help of the antibody anti-Ki67 the proliferative activity in the carcinoma of the digestive tract and have remarked the fact that the proliferative index has been smaller in gastric carcinoma (24.8%) than in the esophageal ones (35.7%), of the colon (37.6%) and the rectum (34.3%). The proliferative index was not correlated to the stage of TNM, tumoral grading, the volume of the tumor, the topography of lesions and not even to the age and sex of the patients. There have been remarked significant statistic variations of proliferative activity in various areas of the same tumour and a certain heterogeneity of the Ki67 expression in the different tumoral stages [78].

Oya M et al. [79] investigating immunohistochemical with Ki67 the metastatic potential of gastric intramucosal carcinoma (943 cases) found a proliferative index of 47.5% for the well differentiated forms presenting lymph nodes involvement, towards the 39.2% in the well differentiated forms lacking lymph nodes metastases. The authors have concluded that the lymph nodes metastasis of the intramucosal forms of gastric carcinoma seems to be correlated to the size of the tumour, the presence of the poorly differentiated components, and particularly for the well differentiated ones and with the index of cellular proliferation [79].

Victorzon M et al. [80] have investigated the prognostic role of the expression of Ki67 and of the fraction of phase S in gastric carcinoma. The authors have noted a high proliferative index at Ki67 correlated to the intestinal type of gastric carcinoma, masculine sex, but very little with the ploidy and the fraction of phase S. Besides it has also been noticed the fact that there were no significant differences concerning the surviving among the patients with tumors with proliferative index at Ki67 big or small, both in the univariance analysis as in those of static multivariance. Thus in gastric carcinoma the immunoreactivity at Ki67 does not bring prognostic information supplementary to the flow cytometry investigation of the content of ADN of tumoral cells [80].

Ramires M et al. [81], investigating the proliferative activity in gastric carcinoma in 43 carcinoma (24 diffuse and 19 of an intestinal type) have noted the fact that the proliferative index at Ki67 in gastric diffuse carcinoma was not significantly different to those of an intestinal type (36.5 versus 28.2). However, the proliferative index has been significantly bigger in superficial areas than in the profound ones (41.9 versus 29.7), no matter the histological type. There was not noted any relations between the proliferative index and the depth of the invasion in the gastric wall, lymph nodes metastasizing, vascular invasion or ploidy [83].

Broll R et al. [82], have investigated with the help of MIB1 the proliferative cellular index in 94 of the gastric adenocarcinoma and have remarked a total index of 47.2% a little bit higher in the center of the tumors (49.1%) to the periphery (44.7%). Surprisingly enough in lymph nodes metastases the index has been decreased than in the primary tumor (39.5% in the first ganglionar station and 33.6% in the second station). However, the tumors with metastasis at a distance have presented a proliferative index higher than those without metastases (55.1% as compared to 44.3%).

In addition, the proliferative index has increased from the well-differentiated forms to the poorly differentiated ones, while the intestinal type has a lower index as compared to the diffuse one. The authors finish their study with the conclusion that the proliferative index does not have any prognostic relevance in gastric carcinoma [82].

Forones NM et al. [83], investigating immunohistochemical 22 cases of gastric carcinoma and namely 22 cases of intestinal metaplasia has noted a 0.4 ± 0.17 Ki67 proliferative index for intestinal metaplasia and namely 0.54 ± 0.19. Moreover, there has been noticed that the expression of Ki67 and bcl-2 was similar in the tumoral cases p53+ or p53-. There were not noted significant differences of the expression of Ki67 connected to the sex of the patients. This indicates an increase of proliferative activity in carcinoma versus intestinal metaplasia.

The expression of p53 was negative in all of the cases with intestinal metaplasia and positive in 68% of the cases of gastric carcinoma. In addition, positivity at p53 was present in all of the cases with an advanced study of the disease (IV) and of only 40% in the cases of gastric carcinoma found in phase I. This fact comes to confirm the fact that the mutation of the oncogene p53 is a late event that takes place in the advanced stages of cancer [83].

The CD34 immunostaining

The expression marker of CD34 has been noticed in all of the cases of adenocarcinoma of upper gastric pole investigated immunohistochemical. The intensity of the immunostaining has been variable from one case to another and in the middle of the same tumor, from one field to another. The most powerful immunostaining has been obvious in the poorly differentiated and diffuse forms of adenocarcinoma, and the intensity has been a moderate one (present in 53.12% of the investigated cases).
Reported to the cases of gastric adenocarcinoma of inferior pole the intensity has been a small one, the majority of these cases presenting score 3 of intensity (55% of the investigated lesions).

Referring to the type of encountered vessels, we have noted the presence of all the maturity vessels from isolated endothelial cells, to small and big vessels, some of them branched. In what vascular microdensity is concerned we have noted the obtaining of a smaller value than in the case of adenocarcinoma of inferior gastric pole and this because of the bigger number of diffuse adenocarcinoma and of mucinous forms in this localization.

The value of vascular microdensity has been variable according to the histopathological type and degree, the highest scores being registered in the cases of poorly differentiated adenocarcinoma and in the only degree, the highest scores being registered in the cases variable according to the histopathological type and localization.

Moreover, vascular microdensity has been bigger in the diffuse forms of gastric pole and this because of the bigger number of diffuse adenocarcinoma and of mucinous forms in this localization.

The value of vascular microdensity has been variable according to the histopathological type and degree, the highest scores being registered in the cases of poorly differentiated adenocarcinoma and in the only degree, the highest scores being registered in the cases variable according to the histopathological type and localization.

The biggest apoptotic index has been noted in grades G1 and G2 and in the tumors with a proliferative cellular index of over 29.77%.

There was not noted the existence of a direct correlation between the apoptotic index and the histologic subtype, the status of lymphatic invasion, the p53 expression or the immunoreactivity at CD34 [86].

Ding S et al. [87] have investigated the expression of CD34 and CD105 in gastric carcinoma, chronic gastritis and hyperplasic polyps in order to establish some clinico-morphological correlations. Thus, the authors have noted a CD105 immunostaining extremely weak in gastric benign lesions, vascular microdensity (MVD) being also reduced, in exchange the expression of CD34 has been higher in these benign lesions. In gastric carcinoma, the expression of CD105 was more intensely reported to the immunoreactivity from the benign lesions. Correlative analysis have shown that vascular microdensity determined through the expressing CD105 are correlated to vascular invasion, distance metastasis and the development of ascites.

Surviving analyses have shown the existence of some reverse correlations between MVD at CD105 and the surviving rate of the sickness. The multivariance analyses have confirmed the fact that MVD at CD105 is an independent prognostic for survival. MVD at CD34 was reversibly correlated to survival but it was not also correlated to other morphoclinical parameters, excepting the formation of ascites.

As a conclusion, CD34 is universally expressed at the level of blood vessels both in benign lesions as well as in the malignant ones while the expression of CD105 has been minimal in benign lesions but intense in the malignant ones. So, both CD105 and CD34 may be used in quantitative and qualitative determinations of angiogenesis, but marker CD105 is being preferred when one has to investigate the prognostic of gastric carcinoma.

Conclusions

The cytokeratinic immunostaining CK7/CK19 confirms the epithelial origin of gastric tumors and namely from the unistratified epithelia. The CK7 is much more intensely expressed in tubular and papillary forms contrastively with “signet ring cell” or mucinous adenocarcinoma type of upper gastric pole. CK19 considered the pan-adenocarcinomatous keratin is over-expressed in gastric adenocarcinoma of intestinal type.

The antigen EMA, marker of the secretory epithelium has been less sensitive in the adenocarcinoma of upper gastric pole as compared to the cytokeratinic and carinoembryonic (CEA) immunostaining, but is useful in the differential diagnosis of lymph nodes metastases in which there is not yet known the exactly origin of primary tumors.

The antigen CEA is intensely expressed in poorly differentiated forms of gastric adenocarcinoma, fact that makes useful the usage of this marker as antibody of first line in the differential diagnosis of poorly differentiated gastric adenocarcinoma by other types of carcinoma. In addition, the marker is useful in the differentiation of intestinal types of adenocarcinoma of the purely gastric ones.
The expression of this marker seems to be correlated to contract no. 190, and it represents the subject of the PhD stages of oncogene invasion in gastric cancer, CEEX research grant “Endoscope, histological and in the diffuse and poorly differentiated forms of overexpress in the advanced stages of the disease, intestinal forms of adenocarcinoma. Dissemination, in the poorly differentiated and the adenocarcinoma is Ki67 whose expression is elevated differentiated intestinal type of gastric adenocarcinoma. There is also an overexpression especially in the well- especially in those with lymph nodes metastases. overexpress in the advanced forms of the disease, malignant neoplasia with mesenchymal origin. The primary tumor is not known, allowing the excluding of the stage of the disease, making possible in this way its being considered as prognostic factor. The vimentin immunostaining proves its utility only in the poorly differentiated and undifferentiated forms, as well as in regional lymph nodes metastasis whose primary tumor is not known, allowing the excluding of malignant neoplasia with mesenchymal origin.

Protein p53 is a useful prognostic marker, being overexpress in the advanced forms of the disease, especially in those with lymph nodes metastases. There is also an overexpression especially in the well-differentiated intestinal type of gastric adenocarcinoma. Another marker of prognostic in the gastric adenocarcinoma is Ki67 whose expression is elevated especially in the advanced forms of cancer with distant dissemination, in the poorly differentiated and the intestinal forms of adenocarcinoma.

The investigated degree of vascularization with CD34 is also a prognostic factor, this marker being overexpress in the advanced stages of the disease, in the diffuse and poorly differentiated forms of adenocarcinoma.

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