E-cadherin in gastric carcinomas related to histological prognostic parameters

DIANA STĂNCULESCU1), CL. MĂRGĂRITESCĂ2), A. STEPAN2), ANCA OANA MITRUT1)

1)PhD student
2)Department of Pathology
University of Medicine and Pharmacy of Craiova

Abstract
Aim: The immunohistochemical study of E-cadherin in gastric carcinomas, related to tumor aggressiveness factors (invasive and metastatic potential). Materials and Methods: The studied material was gastric resection specimens taken from of 60 patients with gastric cancer, during 2009. The tissue was processed using standard histopathological technique, which allowed the assessment of the well-known morphological parameters of prognostic value. Later on the specimens has undergone immunohistochemical processing for E-cadherin (NCH-38 clone), to evaluate its expression in relation with these prognostic parameters. Results: E-cadherin was positive in 65% from gastric carcinomas, with highest positivity index for well (80% cases) and moderate (17.64% cases) differentiated intestinal type tumors, while a large number of poorly differentiated tumors (55.55%) were E-cadherin negative. Among diffuse type carcinomas, the majority of advanced stage tumors (50% of serosal invasive tumors and 100% of peritoneal disseminated tumors) and also a high number of tumors with vascular and lymphatic invasion (50% and respective 80% cases) represented the E-cadherin negative category (54.54%). The E-cadherin staining was also negative in 75% of lymph node positive diffuse type carcinomas and in all metastatic tumors. Conclusions: We found that irrespective of histologic type, the E-cadherin expression was reduced to negativity in advanced stages of gastric carcinoma.

Keywords: gastric carcinomas, prognostic indicators, E-cadherin.

Introduction
Worldwide, gastric cancer ranks fourth in frequency and second in cancer mortality rate; the general evolution of patients with such tumors remains unfavorable, with a 5-year survival rate not exceeding 30% in Western countries [1, 2].

Histologically, gastric carcinomas are classified into intestinal and diffuse types according to Lauren’s classification system, system that is accepted by the AJCC (American Joint Committee on Cancer) and that has the advantage of correlation between histological types, epidemiological features [3, 4].

Pathogenesis of gastric cancer is a classic example of the interaction between genetic and environmental factors, involving infection with Helicobacter pylori as the major risk factor, regardless of histological type of gastric cancer [5]. E-cadherin underexpression and/or altered expression of cell-to-cell adhesion complex (E-cadherin – catenin) found in the context of infection with Helicobacter pylori are considered early events in the development of gastric cancer [6, 7].

E-cadherin, the major adhesion molecule that belongs to the superfamily of calcium-mediated membrane glycoproteins is considered tumor suppressor gene with a well-known role in the development of gastric cancer, acting as a suppressor of invasion [8, 9].

The abnormal expression of E-cadherin was found in many types of cancers including gastric carcinomas, its reduced expression being related to tumor invasive growth and metastatic ability, hence the assumption that loss of E-cadherin-mediated cell-cell adhesion is a prerequisite for tumor cell invasion and metastasis formation [10–12].

According to recent studies, the role of E-cadherin in carcinogenesis does not limit only to invasion and metastasis this molecule being apparently involved in modulating of intracellular signaling, and thus promoting tumor growth. This is based on the mutations of E-cadherin gene found in familial gastric cancer suggesting the E-cadherin involvement in earlier stages of tumor genesis and its role as tumor suppressor gene [13, 14].

Altered E-cadherin expression due to genetic mutations is frequent found in diffuse type gastric carcinomas suggesting the particular importance of genetic mechanisms in developing this type of tumors; beside, inactivated E-cadherin in early diffuse type tumors suggest a role in tumor suppression (in addition to well known role of invasion suppression) of that molecule [15, 16]. While the loss of E-cadherin gene (CDH1) expression was related with susceptibility to diffuse-type gastric cancer, germ line mutations with CDH1 inactivation characterizing hereditary diffuse gastric cancer syndrome [17–19].

Materials and Methods
The studied material represented by gastric tissue, was taken from 60 patients undergoing gastric resection
in Surgery Clinics, Emergency County Hospital of Craiova, during 2009, and diagnosed with gastric carcinomas in Pathology Laboratory in the same hospital. The tissue fragments were fixed in formalin 10% and processed by standard histopathological technique with Hematoxylin–Eosin staining. Cases were classified and interpreted according to Lauren’s classification and evaluation protocol recommended by AJCC (American Joint Committee on Cancer) [3, 20]. We aimed to investigate the morphological parameters of prognostic value: histologic type and tumor grading, depth of parietal invasion, vascular and lymphatic invasion, peritoneal dissemination, lymph node metastases and distant metastases.

The selected cases were subject to further immunohistochemical investigation, using the immunoenzymatic technique with soluble complexes (LSAB/HRP) and DAKO LSAB 2 (System HRP (Universal Dako Labeled Streptavidin Biotin 2 System Horseradish Peroxidase) kit; as a anti E-cadherin primary antibody we used the NCH-38 clone, 1:50 dilution, 30 minutes at room temperature. The tissue sections were previously subjected to microwave antigen unmasking in Tris-EDTA buffer solution pH9; endogenous peroxidase was blocked with 3% hydrogen peroxide and the blocking of nonspecific binding sites was performed with PBS buffer solution that contained saline BSA 8%. The developing of reactions were made with DAB (3,3′-diaminobenzidine). In addition to validating, the reactions were used negative external controls (in which the primary antibody was missed) and positive external controls (cases of well-differentiated oral squamous cell carcinoma).

E-cadherin staining assessment was performed by semi-quantitative analysis, examining at least 10 high power fields of 400× from each tumor. First we evaluated the intensity of reaction using an qualitative score: (intensity score: 0 – negative, 1+ – weak (light brown) 2+ – moderate (brown), 3+ – strong (intense brown) 2+ – moderate (brown), 3+ – strong (intense brown)) and then we evaluate the proportion of positive cells using a quantitative score (% positive cells): 0 – negative, 1 – ≤25%, 2 – 25–50%, 3 – 50–75% 4 – ≥75%). The index of positivity (IP) was calculate by multiply the two scores for each tumor, obtaining an scale with five degrees, in the range 0–12 as follows: IP=0 – negative immunoreactivity (-); IP=1–4 – very low immunopositivity (+); IP=5–8 – low immunopositivity (++); IP=9–12 – high immunopositivity (+++).

### Results

The immunohistochemical analyzed gastric carcinomas were from 60 patients, 39 men and 21 women, aged between 35 and 87 years. Histologically, these 60 cases corresponded to 49 intestinal type carcinomas (81.66%) of which five well differentiated, 17 moderately differentiated and 27 poorly differentiated and to 11 cases, diffuse type gastric carcinomas (18.33%). In intestinal type carcinomas, two of the three tumors with submucosal limited invasion were well-differentiated, while serous invasion (present in 18 of intestinal type carcinomas) and peritoneal dissemination (15 cases) was more frequent in poorly differentiated tumors (66.66% and 60%, respectively). Vascular invasion (seven from 10 cases) and lymphatic invasion (12 from 13 cases) were also more frequent in poorly differentiated tumors as were lymph node involvement (24 from 37 cases) and distant metastases (four from five cases). The diffuse type carcinomas were more frequently tumors with serosal invasion and tumors with peritoneal dissemination (equally, 36.36% each) and were associated with vascular invasion, lymphatic invasion, lymph node involvement and distant metastases in 18.18%, 45.45%, 72.72% and respectively 18.18% cases.

Analysis of E-cadherin expression for the 60 studied gastric carcinomas revealed immunoreactivity with variable index of positivity in 65% of them, including 34 tumors of intestinal type (69.38%) and 45% of diffuse type carcinomas (five cases) and the absence of this marker in the rest of the 21 cases (35%), 15 (30.61%) intestinal-type carcinomas (poorly differentiated tumors, in all cases of) and six (54.54%) diffuse type carcinomas (Table 1).

### Table 1 – E-cadherin expression in gastric carcinomas in relation with histological parameters

<table>
<thead>
<tr>
<th>Histopathological parameters / E-cadherin staining</th>
<th>Histopathological parameters</th>
<th>Intestinal type carcinomas</th>
<th>Diffuse type carcinomas</th>
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<tr>
<td>E-cadherin expression</td>
<td>Well-differentiated</td>
<td>Moderate differentiated</td>
<td>Poorly differentiated</td>
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<td>Submucosal invasion</td>
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<td>Muscular invasion</td>
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<td>Serosal invasion</td>
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<td>Peritoneal dissemination</td>
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<td>Total cases</td>
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<tr>
<td>Vascular invasion</td>
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<td>Lymphatic invasion</td>
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<td>Lymph node metastases</td>
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<td>Distant metastases</td>
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- Well-differentiated intestinal type carcinomas characterized by the formation of distinct malignant glandular structures with submucosal (two cases) and muscular (three cases) invasion showed intense
E-cadherin immunoreactivity (IP++, range 9–12) in four of the five cases (80%) and low immunoreactivity (IP++, range 4–8) in only one case, represented by one (20%) of tumors with muscular invasion.

Intense E-cadherin immunopositive well-differentiated carcinomas were characterized by continuously and uniformly intense brown epithelial membranar staining, involving the malignant glands in most tumor mass (Figure 1).

E-cadherin expression was positive in all three carcinomas with locoregional lymph node metastases observing the reduced expression (IP++) in tumor with the highest number of positive lymph nodes.

Among the moderately differentiated intestinal-type carcinoma E-cadherin was intense immunopositive (IP+++, range 9–12) in only three of the 17 evaluated tumors (17.64%) in the rest of the other 14 cases (82.35%), observing the reduced expression of E-cadherin both in intensity and as % positive cells in tumor mass (IP++, range 5–8) (Figure 2).

Reduced expression of E-cadherin (IP++) was found for tumors with deeper parietal invasion including those two tumors with muscle invasion and the serous invasive tumors (six cases) and also the six tumors associated with peritoneal dissemination (Figure 3).

E-cadherin immunopositivity was also reduced (IP++, range 5–8) in all tumors with vascular (three cases) and lymphatic (one case) invasion. Related to the loco-regional lymph nodes status, the E-cadherin expression was intense (IP+++) in three of the four moderately differentiated carcinomas without lymph node metastases, lymph nodes involvement and liver metastases (one case) being correlated with reduced expression of this marker (IP++).

Among the 27 poorly differentiated intestinal-type carcinomas, E-cadherin expression was in very low immunopositivity range (IP+, 1–4) in 12 cases (44.44%), while the rest of the other 15 cases were immunonegative (IP) (55.55%).

Poorly differentiated tumors with very low E-cadherin expression (PI+) were characterized by a continuous membranar but low intensity (light-brown) staining, or conversely, discontinuous, fragmented, but moderate intensity (brown spotted) staining in most cases (seven cases, 58.33%) affecting ≤25% of the tumor cells (Figures 4 and 5).

E-cadherin negative poorly differentiated carcinomas category (PI-) included most of the serosal invasive tumors (8/12 cases, 66.66%) and also most of tumors with adjacent organs/tissues involvement (7/9 cases, 77.77%). Similarly, vascular and lymphatic invasion were more frequently in E-cadherin immunonegative tumors (IP-) with a number of five from seven cases (71.42%) for tumors with vascular invasion and 10 of 12 cases (83.33%) for those with lymphatic invasion. Poorly differentiated carcinomas with locoregional lymph node metastases were about equally very low immunopositive (PI+) (13/24 cases, 54.16%) or immunonegative (IP) for E-cadherin. E-cadherin expression was negative (IP-) in three of the four poorly differentiated carcinomas with distant metastases and fell to the lower limit of very low immunopositivity range (IP+, value 1) in the other tumor.

• Diffuse type gastric carcinomas were E-cadherin immunonegative (IP-) in six of the 11 tumors (54.54%) evaluated for this marker. In the other five tumors (45.45%), E-cadherin immunopositivity index was very low (IP+, range 1–4), observing weak or moderate intensity staining in up to 50% of tumor cells; the highest level of E-cadherin expression (IP=4, the upper level of very low immunopositivity) was found in that only tumor with submucosal invasion assessed (Figure 6).

Those six E-cadherin immunonegative diffuse type carcinomas corresponded to two of the four serosal invasive tumors (50%) and to the four tumors with peritoneal dissemination. Those tumors with vascular invasion (two cases) were equally E-cadherin very low immunopositive (IP+) or immunonegative (IP-) while the lymphatic invasion prevailed between immunonegative tumors (IP-) (4/5 evaluated cases, respectively 80%). Most of diffuse type carcinomas associated with lymph node metastases (six of eight cases, 75%) and those two tumors with distant metastases were E-cadherin immunonegative.
For the 60 gastric carcinomas included in our study, we evaluated the relation between the E-cadherin expression and the well-known prognostic factors including histologic type and tumor grading, depth of parietal invasion, vascular and lymphatic invasion, peritoneal dissemination, lymph node metastases and distant metastases. Among these gastric carcinomas predominated the intestinal type ones (81.66%), especially poorly differentiated (55.10%), tumors found frequently in advanced stage (with serosal invasion and peritoneal dissemination, summing 63.63% cases); the rest of 18.33% gastric carcinomas were of diffuse type. Among the histological parameters of poor prognosis were particularly noted the lymphatic invasion (18 of a total of 60 cases) and lymph node metastases (45 of a total of 60 cases), frequently associated with intestinal type carcinoma poorly differentiated (44.44% and 88.88% respectively) and diffuse type carcinomas (45.45% and 72.72%, respectively).

Analyzing the results of E-cadherin expression, we found positive immunoreactivity in 65% of gastric carcinomas, mainly intestinal-type tumors (69.38%) compared with only 45% of the diffuse type tumors. We have also noted the negative staining in the remaining 30.61% of intestinal type carcinomas (all poorly differentiated tumors) and 54.54% of the diffuse type carcinomas (Figure 7). In agreement with these results, similar studies reported variable decrease (between 17% and 92%) of E-cadherin expression in gastric carcinomas (compared with normal non-neoplastic gastric mucosa), mainly for poorly differentiated intestinal-type tumors and diffuse type carcinomas [13, 21–23].

For the 69.38% of the intestinal type carcinomas that expressed E-cadherin, the immunopositivity was intense in majority of well-differentiated tumors (80%) and
only in 17.64% of moderately differentiated tumors. In contrast, in poorly differentiated carcinomas the E-cadherin staining was very low in 44.44% cases and negative for the rest of 55.55% tumors (Figure 7). This direct relationship between E-cadherin expression and degree of differentiation of intestinal type carcinoma, revealed in our study by intense and uniform membranar staining at cell-cell interface throughout the tumor mass consisting of distinct glandular structures, in majority of well-differentiated tumors, and also by low-intensity expression in limited tumor areas or no expression in poorly differentiated carcinomas is well documented in the literature [13, 21, 24].

Among intestinal-type carcinoma with deep peritumoral invasion (serosal involvement) E-cadherin was expressed with low immunopositivity in moderately differentiated tumors (22.22% of cases); in poorly differentiated tumors the immunoexpression of E-cadherin was very low (22.22% of cases) or negative (66.66%). Most of tumors with peritoneal dissemination showed very low E-cadherin immunopositivity or negative staining (summing 60% cases), all of them being poorly differentiated (Figure 7). These findings overlap the results of similar studies reporting the marked reduction to negativity of E-cadherin expression in advanced tumors, loss of its expression being essential for tumor invasiveness [13, 21].

Generally, E-cadherin is high expressed in well-differentiated cancers that maintain cell-cell adhesiveness and are less invasive while the expression of this molecule is reduced in poorly differentiated tumors that have lost their intercellular adhesion in parallel with the acquisition of high invasive potential [11]. Reduced cell-cell adhesiveness in carcinomas is reflected in the architectural disorder characteristic of malignant tumors: the tumor cells are dyscohesive throughout the entire tumor mass in diffuse-type cancers, whereas in those solid tumors with high metastatic potentials they are often focally dissociated or dedifferentiated at the invasive front [25].

An interesting aspect to note is that in human cancers seem to occur both irreversible and reversible mechanisms for inactivating the cell-cell adhesion system, demonstrated by the re-establishment of the functionality of cadherins systems, e.g. through forced induction of E-cadherin expression in cultured tumor cells resulting in the reversion from an invasive, mesenchymal, to a benign, epithelial phenotype, which would explain the tumor cells growth with secondary tumors: the tumor cells are dyscohesive throughout the entire tumor mass in diffuse-type cancers, whereas in those solid tumors with high metastatic potentials they are often focally dissociated or dedifferentiated at the invasive front [25].

Vascular and lymphatic invasion were predominant between E-cadherin negative intestinal-type tumors (50% and 83.33%) literature studies confirming the relationship between reduced/absent E-cadherin expression in such tumors or, conversely, being in contravention of these results [21, 24, 26].

E-cadherin expression was preserved in 67.5% of intestinal-type carcinoma associated with lymph node metastases, with a variable immunopositivity index (high in 5%, low, in 35% and very low, in 27.5% of cases, noting the reduced frequency of tumors with intense immunostaining). The E-cadherin was negative in the rest of the other 32.5% of cases, observing at the same time its reduced expression to negativity in parallel with the increase in number of positive lymph nodes, observation found in similar studies [21, 24] (Figure 7). Distinct metastases were more frequent in E-cadherin negative intestinal type tumors (60%), most of them poorly differentiated. The reduced expression of E-cadherin was related with infiltrative and metastatic potential of gastric cancer, on the one hand, although, on the other hand, there are studies supporting that the tumor development in metastatic sites requires the preservation of E-cadherin expression [26, 27].

In diffuse type gastric carcinomas, we observed very low levels of E-cadherin immunopositivity in 45.45% of cases and also the absence of its expression in the rest of the other 54.54% of cases, results that are consistent with those of similar studies [24, 28]. Absence of E-cadherin expression, known as indicator of cellular cohesion loss and invasiveness was found with increased frequency between advanced diffuse type carcinomas (half of serosal invasive tumors and all cases with peritoneal dissemination) and also among those tumors with vascular and lymphatic invasion (50% and 80% cases respectively) [28]; E-cadherin expression was also negative in the majority of diffuse type gastric tumors associated with lymph node metastases (75%) and in all metastasized tumors (Figure 7).

**Conclusions**

In our study, E-cadherin was intensely expressed more frequently in gastric carcinomas of intestinal type and well differentiated while poorly differentiated tumors and diffuse type carcinomas demonstrated a high rate of negative reactivity. Irrespective of histological type, we found reduced E-cadherin immunopositivity in advanced stages of disease with an inverse relation between E-cadherin expression and adverse prognostic parameters (including depth of parietal invasion, vascular and lymphatic invasion and lymph node metastasis).

**References**


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Corresponding author
Diana Stânculescu, PhD candidate, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania; Phone/Fax +40251–599 228, e-mail: seddiana@yahoo.com