Expression of E-cadherin in lung carcinoma, other than those with small cells (NSCLC)

MIRELA LOREDANA GRIGORAȘ1, TEODORA SMARANDA ARGHIRESCU2, ROXANA FOLESCU1, IOANA CRISTINA TALPOS3, CPIRIAN MIHAI GÎNDAC4, CARMEN LĂCĂMĂIOARA ZAMFIR5, MĂRIOA CORNIANU6, MIRELLA DORINA ANGHEL3, CODRINA MIHAELA LEVAȚ7

1) Discipline of Anatomy and Embryology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania
2) Discipline of Pediatrics, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania
3) Discipline of Oro-dental Diagnosis and Ergonomics, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania
4) Discipline of Anesthesia and Intensive Care, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania; Clinic of Anesthesia and Intensive Care, "Pius Brâncuşi" Emergency County Hospital, Timișoara, Romania
5) Discipline of Histology, Department of Morpho-Functional Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania
6) Discipline of Morphopathology, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania
7) Legal Department, "Victor Babeș" University of Medicine and Pharmacy, Timișoara, Romania

Abstract

It was suggested that the decrease and/or loss of E-cadherin expression in non-small cell lung cancer (NSCLC) is responsible for the development of the malignant phenotype. Moreover, clinical studies showed that the reduced expression of E-cadherin is associated with tumoral differentiation, with the presence of lymph node metastasis and with unfavorable diagnosis of patients with NSCLC. In order to evaluate if E-cadherin expression is involved in the NSCLC pathogenesis and significantly associated with clinicopathological parameters, we investigated the immunohistochemical (IHC) expression of E-cadherin in 47 lung carcinomas with tumoral resection pieces in the control peritumoral lung tissue, looking for possible correlations between the expression of this molecule and the clinicomorphological features and the evolutive development of the malignant phenotype. Moreover, clinical studies showed that the reduced expression of E-cadherin is associated with the unfavorable prognosis of patients with NSCLC – for patients with reduced expression of the E-cadherin/α-catenin complex, needing chemotherapy or radiotherapy.

Keywords: E-cadherin, NSCLC, lung carcinoma, lymph node metastasis, prognosis.

5 Introduction

E-cadherin (E-cad) is an adhesion molecule of the epithelial cells, dependent on calcium that plays a key role in stabilizing and maintaining intercellular connections [1–4]. E-cadherin is bound to cytoskeleton through catenins, and acts as a suppressor of invasion and tumoral metastasis. E-cadherin is expressed on the surface of cells in the majority of epithelial tissues, and plays an important role in stabilizing cell polarity, in maintaining the epithelial integrity and the cellular differentiation [5–8].

E-cadherin is involved in carcinogenesis, its expression being frequently reduced or absent in human epithelial carcinomas. Losing the intercellular adhesion and the invasion of tumoral cells in the adjacent conjunctive tissue were associated with the malignant phenotype.

Studies showed a reduced protein expression of E-cadherin associated with the reticent prognosis in a variety of human cancers, as well as those of esophagus, stomach, bowel, liver, pancreas and bladder, showing that the decrease and/or the absence of E-cadherin expression in carcinomas are positively linked with the potential of invasion and metastasis of these tumors, being connected to the tumoral progression and metastasis [9].

It was suggested that the decrease and/or the loss of E-cadherin expression in non-small cell lung cancer (NSCLC) was responsible for the development of the malignant phenotype. Moreover, clinical studies showed that the reduced E-cadherin expression is associated with tumoral differentiation, with lymph node metastasis, and with the unfavorable prognosis of patients with NSCLC [10].

Our aim was to implement a flexible, personalized approach, targeted in function of the etiopathogenic and genotypic aspects, in order to offer an integrative, complex research [11–18]. The attitude must take, with great attention, into account, all the present signs, symptoms and associated pathologies, the medical and family history and the heredo-collateral antecedents [19–21].

The aspects concerning the potential adverse events, quality of life and vulnerabilities, must be carefully approached [18, 22, 23]. The risk and resilience factors must be known and evaluated and the therapeutic approach must be ethical with the lowest number of adverse events encountered [24–27].

Relatively little research has been conducted on the expression of E-cadherin in lung carcinoma – NSCLC,
other than those with small cells. Our present study is very useful, original and of great impact, because it makes significant correlations in this field, between the E-cadherin expression and the prognosis of NSCLC. Further studies are needed, in order to offer a complex integrative proper image.

To our knowledge, this type of studies correlating these prognostic markers, are also in Romania rare. The study includes a large cohort of patients. This is an important aspect, as until now there is a lack even of international studies, concerning these aspects in the population and the topic is of great interest.

**Patients, Materials and Methods**

The E-cadherin expression was evaluated on tissular sections from 47 patients (40 men and seven women), under sanatorium surgical resection for lung carcinoma. The study period was between 2009–2014, the histopathological identification of lesions was done according to the 2011 International Association for the Study of Lung Cancer/AmERICAN Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) lung cancer classification. The patients’ ages varied between 42 and 69 years old, with an average age of 59.5 years. The medical charts of the 47 patients were revised retrospectively in order to gather pieces of information about the clinicopathological parameters, such as age, smoking history, histological degree, and TNM stage. None of the patients included in the study were treated with chemotherapy or radiotherapy before the surgery.

Postoperatively, the patients were under supervision for a period of time between one month and 21 months. Death caused by the lung cancer was the terminal event for the survival assessment. The survival period was calculated from the time of surgery until death or until last exemption.

The tissular material resected through surgery (containing the tumor and the adjacent non-tumoral lung tissue) was put in 10% formalin and included in paraffin, and then sectioned to 4 μm thickness and customary stained with Hematoxylin–Eosin (HE). Histologically, we identified 22 keratinized and non-keratinized squamous cell carcinoma (SCC), 16 pulmonary adenocarcinoma (ADC) [four acinar ADC, one papillary ADC, one solid carcinoma with mucus, six combined ADC, and four bronchioloalveolar carcinoma (BAC)], and nine large cell carcinoma (LCC).

**Immunohistochemistry**

For immunohistochemical (IHC) expression staining of E-cadherin, we used the Labeled Streptavidin–Biotin (LSAB) technique on tissular sections added in formalin and included in paraffin. Deparaaffinized sections (with 4 μm thickness) were treated with 3% hydrogen peroxide \( (\text{H}_2\text{O}_2) \) for 10 minutes, in order to block the endogenous peroxidase, then immersed in 0.05 M citrate buffer (pH 6) and heated for 10 minutes in the microwave, at 100°C, to enhance the recovery of antigen.

The sections were incubated for 30 minutes with anti-E-cadherin rabbit monoclonal primary antibody, clone EP700Y, in 1:50 dilution, and then treated for 20 minutes with LSAB2. To visualize the reaction, the sections were stained with 3,3’-diaminobenzidine (DAB) tetrahydrochloride, followed by light counterstaining of nuclei with Mayer’s Hematoxylin.

The normal bronchial epithelium adjacent to the tumor was used as positive check for the evaluation of E-cadherin expression. For the negative check of reaction, the sections were applied with the same staining procedure, but excluding the primary antibody.

**Evaluation of IHC staining**

Following the treatment of sections with anti-E-cadherin antibody, a membrane staining pattern and/or cytoplasmatic emerged. In order to quantify E-cadherin immunoexpression, we applied the criteria used by Lim et al. (2000) [9]. We considered to be negative (−) E-cadherin immunoreaction, the immunostaining of less than 5% of tumor cells or the absence of membrane expression in tumor cells.

E-cadherin expression was defined as positively weak (+), when between 5% and 50% of the tumor cells were stained, and strongly positive (++), when >50% of the tumor cells became stained. The strongly positive E-cadherin cases (++) were included in the tumor group with “preserved” E-cadherin expression, while the rest (tumors +,−) were included in the tumors with “decreased” E-cadherin expression.

We noticed the following patterns of E-cadherin expression: linear patterns – with even and uninterrupted membrane staining (limited to cellular membrane); spotted pattern – with interrupted immunostaining but limited to cellular membrane; diffuse pattern – when there was a membrane and cytoplasmatic immunostaining. The even staining pattern of the cellular membranes was considered to be normal, and as an abnormal expression of E-cadherin, we considered the negative even and the diffuse patterns (cytoplasmatic and membrane). We did not take into consideration the areas with necrosis and bleeding.

The expression of the non-tumoral lung tissue served as internal check. For every case, the IHC expression of the E-cadherin from malignant cells was compared to the intensity of the immunoreaction in the normal bronchial epithelium, having an intense and homogeneous E-cadherin expression at the membrane level.

In order to analyze the existence of a possible correlation between the E-cadherin expression and the proliferative activity of lung carcinomas, estimated through Ki-67 mitotic index (MI), the IHC expression of Ki-67 antigen was evaluated by using anti-Ki-67 monoclonal antibody (clone MIB-1, Dako, Carpinteria, CA, USA).

High Ki-67 activity, meaning increased proliferative expression, was considered for cut-off values >50% and reduced activity <20%.

**Statistical analysis**

The correlations between E-cadherin expression and the clinicopathological factors, and the relation between E-cadherin expression and proliferative activity (appreciated through the evaluation of Ki-67) were statistically analyzed with the \( \chi^2 \) (chi-square) test function. The value of \( p<0.05 \) was considered to have a statistical significance.
Results

Thirty-seven (78.72%) of the 47 NSCLC examined cases showed reduced or absent immunoreaction for E-cadherin. We noted a reduced expression of E-cadherin in 19 of the 22 SCC (86.36%), in 10 of 16 ADC (62.5%) (two acinar ADC, eight BAC) and eight of nine (88.89%) LCC (Table 1).

We did not notice a significant relation between E-cadherin expression and the histological type of cancer. Both in SCC and ADC, the frequency of cases with reduced E-cadherin expression was higher (86.36% in SCC; 62.5% in ADC) than the preserved type (13.64% in SCC; 37.5% in ADC) (p=0.087) (Table 2).

The frequency of linear pattern (13.64% in SCC; 6.25% in ADC) was significantly lower than the spotted pattern (22.72%; 25%) or diffuse pattern (50%; 43.75%) (p=0.087). In general, positive E-cadherin tumors presented a membrane or spotted pattern, while the tumors with reduced expression of E-cadherin had a diffuse pattern or a weak cytoplasmatic staining (Table 3).

Table 1 – The relation between E-cadherin expression and clinicopathological factors in NSCLC

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>No. of cases</th>
<th>Expression of E-cadherin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>20</td>
<td>Preserved n (%)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>27</td>
<td>Decreased n (%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III–IV</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological degree</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1–G2</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3–G4</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCC</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma; LCC: Large cell carcinoma; n: No. of cases; p-value from χ² (chi-square) test; Value of p between SCC, ADC and LCC.

Table 2 – E-cadherin expression according to the histological type in NSCLC

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. of cases</th>
<th>Preserved E-cadherin expression</th>
<th>Decreased E-cadherin expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC</td>
<td>22</td>
<td>++</td>
<td>13.64%</td>
<td></td>
</tr>
<tr>
<td>ADC</td>
<td>16</td>
<td>+– Subtotal</td>
<td>37.5%</td>
<td></td>
</tr>
</tbody>
</table>

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; ADC: Adenocarcinoma; Value of p between SCC and ADC.

Table 3 – E-cadherin expression pattern correlated with the histological type of NSCLC

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Linear pattern</th>
<th>Spotted pattern</th>
<th>Diffuse pattern</th>
<th>Negative E-cadherin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC (n=22)</td>
<td>3 (13.64%)</td>
<td>5 (22.72%)</td>
<td>11 (50%)</td>
<td>3 (13.64%)</td>
<td></td>
</tr>
<tr>
<td>ADC (n=16)</td>
<td>1 (6.25%)</td>
<td>4 (25%)</td>
<td>7 (43.75%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

SCC – squamous cell carcinoma; ADC – adenocarcinoma; n: No. of cases.

We noticed a distinct membrane staining in SCC (Figure 1, a–f), sometimes more obvious in central areas of lobules and nests of tumoral cells with a better differentiation, in comparison to the marginal areas. We noticed more frequently a staining pattern in spots and diffuse in ADC (Figure 2, a and b) and BAC (Figure 2, c and d), without differences regarding the intensity of immunoreaction in areas with variable histological degree. LCC got stained positively weak, with E-cadherin expression mainly cytoplasmatic (Figure 3, a and b).

E-cadherin expression was more frequently preserved in differentiated tumors (G1–G2), comparatively with weakly differentiated tumors (G3–G4) (25% vs. 17.39%), the correlation between E-cadherin expression and the increase of histological degree being statistically insignificant (p=0.524) (Table 4).

The linear pattern of E-cadherin expression was noticed in well-differentiated cancer cells, while the diffuse or spotted pattern was noticed more frequently in weakly differentiated tumors.

Even though there were not any significant correlations between E-cadherin expression and the majority of clinicopathological factors that we analyzed, such as: age (p=0.853), gender (p=0.134), TNM stage (p=0.439), histological differentiation (p=0.524), and the histology of tumors (p=0.146), the E-cadherin expression still was not significantly decreased in male smokers (p=0.004).

We could not note a significantly reduced expression of E-cadherin for the histological type of SCC, comparing with ADC and LCC (p=0.146).

In patients with reduced expression of E-cadherin, there was an invasion of lymph nodes in 19 of the 24 (79.2%) cases (p=0.004), invasion of pleura in 11 of the 15 (73.3%) cases (p=0.07), invasion of blood vessels in nine of the 14 (64.29%) cases (p=0.285), and invasion of lymphatic vessels in 16 of the 23 (69.57%) cases (p=0.060). The tumoral relapses were noted in six of the seven (85.71%) cases (p=0.058), and remote metastasis in nine of the 11 (81.82%) cases (p=0.034) (Table 5; Figure 4).
Figure 1 – Squamous cell carcinoma: (a and b) Intense membrane E-cadherin expression (++); (c-f) Membrane E-cadherin expression with linear pattern and “in spots”. Anti-E-cadherin antibody immunostaining, ×200.

Figure 2 – (a and b) Lung adenocarcinoma with membrane E-cadherin expression and linear pattern. Anti-E-cadherin antibody immunostaining, ×200.
Expression of E-cadherin in lung carcinoma, other than those with small cells (NSCLC)

Table 4 – E-cadherin expression according to the differentiation degree in NSCLC

<table>
<thead>
<tr>
<th>Differentiation degree</th>
<th>No. of cases</th>
<th>Preserved E-cadherin expression</th>
<th>Decreased E-cadherin expression</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>G1–G2</td>
<td>24</td>
<td>6 (25%)</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>G3–G4</td>
<td>23</td>
<td>4 (17.39%)</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

NSCLC: Non-small cell lung cancer; p-value from χ² (chi)-square test.

Table 5 – Relation between E-cadherin expression and clinicopathological factors in NSCLC

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>No. of cases</th>
<th>E-cadherin expression</th>
<th>Preserved n (%)</th>
<th>Decreased n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive lymph nodes</td>
<td>24</td>
<td>5 (20.8%)</td>
<td>19</td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Invasion of pleura</td>
<td>15</td>
<td>4 (26.67%)</td>
<td>11</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>14</td>
<td>5 (35.71%)</td>
<td>9</td>
<td></td>
<td>0.285</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>23</td>
<td>7 (30.43%)</td>
<td>16</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Relapses</td>
<td>7</td>
<td>1 (14.29%)</td>
<td>6</td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>Remote metastasis</td>
<td>11</td>
<td>2 (18.18%)</td>
<td>9</td>
<td></td>
<td>0.034</td>
</tr>
</tbody>
</table>

NSCLC: Non-small cell lung cancer; n: No. of cases; p-value from χ² (chi)-square test.

Figure 2 (continued) – (c and d) Bronchioloalveolar carcinoma: pneumocytes of type II. Anti-E-cadherin antibody immunostaining, ×200.

Figure 3 – Large cell carcinoma with reduced E-cadherin expression (+). Anti-E-cadherin antibody immunostaining, ×200.

Table 6 – Relation between E-cadherin expression and clinicopathological factors in non-small cell lung cancer (NSCLC).

The reduced expression of E-cadherin correlated significantly with the positive lymph nodes (p=0.004) and remote metastasis (p=0.034); also noted was an almost significant correlation between reduced E-cadherin and tumoral relapses (p=0.058) and invasion of pleura (p=0.07).

An almost significant correlation (p=0.089) was noted between the E-cadherin expression and the Ki-67 proliferative activity (Table 6; Figure 5). Twenty-six (70.27%) of the patients group with reduced E-cadherin expression...
and only four (40%) of the group with preserved E-cadherin expression had an increased proliferative expression, being included in NSCLC group with high Ki-67 activity (Table 6; Figure 5).

Table 6 – Relation between E-cadherin expression and ki-67 proliferative activity

<table>
<thead>
<tr>
<th>E-cadherin expression</th>
<th>No. of cases (%)</th>
<th>Ki-67 immunoreactivity</th>
<th>Low Ki-67 MI</th>
<th>Low Ki-67 MI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved</td>
<td>37 (78.73%)</td>
<td>10 (21.27%)</td>
<td>4 (40%)</td>
<td>6 (60%)</td>
<td>0.089</td>
</tr>
<tr>
<td>Reduced</td>
<td>10 (21.27%)</td>
<td>6 (60%)</td>
<td>4 (40%)</td>
<td>10 (21.27%)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5 – Relation between E-cadherin expression and Ki-67 proliferative activity. MI: Mitotic index.

Out of 47 NSCLC of examined E-cadherin expression, the complete information about dispensarization and tracking (over a period of 21 months for those alive) were available for 17 of the patients.

The survival rates of patients with NSCLC were of five months (for a period of tracking between one and 21 months) for patients with reduced E-cadherin expression, and seven months (for a period of tracking between one and 15 months) for those with preserved E-cadherin (Table 7). Even though we noticed no significant difference between the two groups, the patients with reduced E-cadherin expression had a more reserved prognosis than those with preserved expression.

Table 7 – Relation between E-cadherin expression and survival of patients

<table>
<thead>
<tr>
<th>E-cadherin expression</th>
<th>No. of cases (%)</th>
<th>Average rate of survival (tracking period of time) [months]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved</td>
<td>10 (21.27%)</td>
<td>7 (1–16)</td>
</tr>
<tr>
<td>Reduced</td>
<td>37 (78.73%)</td>
<td>5 (1–21)</td>
</tr>
</tbody>
</table>

Discussion

Evaluating the IHC expression of E-cadherin in 65 cases of NSCLC and in the adjacent non-tumoral lung, Myong [28] notes a reduced or absent expression of E-cadherin in 51 (78.4%) of cases and the preservation of E-cadherin expression in 14 (21.6%) of the examined NSCLC. The authors found a significantly decreased expression of E-cadherin in SCC, comparing with ADC (p=0.032), similar results being noted by Lee et al. (2000) [29] and Choi et al. [30]. Based on these observations, we describe a significant relation between E-cadherin expression and histological type of NSCLC, but this does not correlate the reduced expression of E-cadherin with the clinicopathological parameters of prognosis, as well as the histological degree, TNM stage and survival.

According to other studies, SCC have a different pattern of E-cadherin expression compared to ADC [31], Myong [28] suggesting that E-cadherin expression in NSCLC can be used to differentiate SCC from ADC.

Even though the relation between E-cadherin expression and the habit of smoking or male gender is unclear, it can still be supported while SCC (when it has E-cadherin expression relatively decreased) is the main histological type of cancer in male smokers.

Starting from the statement that reduced or absent E-cadherin expression is weakly correlated with the high proliferative activity (appreciated through immunostaining with MIB-1) it was suggested that the decrease of E-cadherin expression is followed by the loss of negative control feedback (determined by the intercellular interaction), enhancing the cellular proliferation [32]. It was recently stated that E-cadherin also has a function of growth suppression, inducing the stop of cellular cycle through the enhancement of a cyclin-dependent kinase inhibitor [33].

Previous studies reported an abnormal E-cadherin expression in 42–81% of lung carcinomas – NSCLC [30, 34], Böhm et al. [31] found reduced or absent E-cadherin immunoreaction in 53% of the cases; E-cadherin expression was considered to be decreased when 90–95% of the tumoral cells became colored, and the coloring intensity was more reduced than the one of the adjacent normal epithelium, and absent when <5% of tumoral cells became colored.

In the study conducted by Lim et al. [9], E-cadherin expression was more frequently preserved in SCC (59%) than in ADC, and in well differentiated tumors, comparatively with the weakly differentiated (60% vs. 25.9%). In NSCLC with lymph node metastasis, E-cadherin expression dropped from 66.3% (≤N1) to 38.6% (≥N2), indicating that the loss of E-cadherin expression is responsible for acquiring the malignant potential of lung cancer, as well as the potential role of E-cadherin in the histological differentiation of lung cancer. Behrens et al. [7] noticed that the inhibition of cadherins with antibodies initiates invasiveness of tumoral cells, and Sulzer et al. [34] sees a high percentage of positive E-cadherin tumor cells in patients without lymph node metastasis (N0, 63%). Böhm et al. [31] found a reduced level of E-cadherin expression in lung cancer, with spotted or diffuse pattern in moderate SCC and weakly differentiated. Using the anti-E-cadherin monoclonal antibody (HECD-1), Oka et al. [35] found a correlation between E-cadherin expression and the degree of tumoral differentiation in gastric cancer, E-cadherin expression being preserved in 70% of the differentiated tumors, and reduced in the majority of undifferentiated tumors (85%).
The results shown in our study are difficult to compare with other studies due to the semi-quantitative criteria of quantification of immunoreaction; compared to the normal tissues, the malignant tumors that were analyzed had a heterogeneous E-cadherin expression.

We found a decreased expression of E-cadherin in 37 (78.4%) and preserved in 10 (21.28%) of the analyzed NSCLC. E-cadherin expression was reduced in male smokers (p=0.004), correlating significantly with lymph node metastasis (p=0.004), and remote (p=0.034), and almost significantly with tumoral relapses (p=0.058), and invasion of pleura (p=0.07), but it did not correlate with age (p=0.853), gender (p=0.134), TNM stage (p=0.439), histological differentiation (p=0.524), and tumor histology (p=0.146). An almost significant correlation (p=0.089) was noticed between E-cadherin expression and the Ki-67 proliferative activity (appreciated by the marking index IM Ki-67). Twenty-six (70.27%) of the patients with reduced expression of E-cadherin, and only four (40%) of those with a preserved E-cadherin expression had a high proliferation rate, being included in the NSCLC group with high Ki-67 MI.

Even though other studies, Chirieac (2016), approach the different histological subtypes of lung cancer in separate study samples, we had an integrative view [36]. The study has its limits because it is difficult to capture all these aspects, also concerning the proliferation index Ki-67 values in different histological subtypes of lung cancer. Some existing studies reported on the prognostic value of Ki-67 index with various endpoint results. No consensus on the prognostic value of Ki-67 was found among the published studies neither according to the stage nor the histological subtype. Ki-67 index seems to be of prognostic influence in NSCLC although largely variable cut-off levels have been used in different studies and a standardization of methodology is required. Further research is needed and several biomarkers in combination may be necessary to more sufficiently stratify patients to various treatment options than is currently possible. The reduced expression of E-cadherin in NSCLC has been captured also in other studies but through our existent study, we put into forefront, interesting aspects and captured also in other studies but through our existent view [36].

The study has its limits because it is difficult to capture all these aspects, also concerning the proliferation index Ki-67 values in different histological subtypes of lung cancer. Some existing studies reported on the prognostic value of Ki-67 index with various endpoint results. No consensus on the prognostic value of Ki-67 was found among the published studies neither according to the stage nor the histological subtype. Ki-67 index seems to be of prognostic influence in NSCLC although largely variable cut-off levels have been used in different studies and a standardization of methodology is required. Further research is needed and several biomarkers in combination may be necessary to more sufficiently stratify patients to various treatment options than is currently possible. The reduced expression of E-cadherin in NSCLC has been captured also in other studies but through our existent study, we put into forefront, interesting aspects and correlations that need further research in the future [28]. On one side, we captured the complexity and heterogeneity of existent situations but on the other side, we want to embrace a personalized, tailored therapeutic approach, deeply interconnected with the clinical information. So, that we observed and analyzed the significant relation between E-cadherin expression and histological type of NSCLC.

The expression of E-cadherin/α-catenin complex was analyzed in NSCLC, a reduced immunoreactivity of E-cadherin being noted in all histological types of lung cancer [SCC, ADC, adenosquamous carcinoma (ASC)]. The decrease of E-cadherin/α-catenin complex expression (more frequently in moderate and weakly differentiated carcinomas) was correlated with the tumoral histological type, stating that the alteration of E-cadherin and α-catenin expressions in NSCLC plays a role in the manifestation of the malignant phenotype [37]. Other authors [31] described the reduced level of E-cadherin and altered patterns in moderate and weakly differentiated SCC, as well as in small cell lung cancer (SCLC), but not in well-differentiated SCC or in ADC.

Hidaka et al. [38] noted a positive expression of E-cadherin in BAC and a reduced E-cadherin expression with disorganized pattern in 71% of ADC. E-cadherin expression was significantly more reduced in BAC with intra-lung metastasis than in BAC without intra-lung metastasis, suggesting that the altered function of E-cadherin and α-catenin can concur to detachment of cancer cells from the primary lesion, with the emergence of intra-lung metastasis.

Other studies described the relation between reduced E-cadherin expression, tumor dedifferentiation, and lymph node metastasis, in patients with NSCLC, the reduced expression of E-cadherin being associated with tumoral dedifferentiation, lymph node metastasis and the reserved prognosis [39].

Similar results were also noted by Böhm et al. [31], showing that the absence of E-cadherin/α-catenin complex expression cannot be correlated with any of the histopathological criterion of epithelial carcinoma. Nawrocki-Raby et al. [40] explained these results through the fact that finding a correlation between the negative adjustment can underline the complexity of cell adhesion system and the importance of examining not only the E-cadherin expression. Also, it was showed that the abnormal cell adhesion can exist in tumoral cell lines with α-catenin deficit, even though these cells show normal quantities of E-cadherin on their surface [41].

Conclusions
Expression of E-cadherin was reduced in most of the studied NSCLC (78.4%), and especially in SCC, known as the most common type of lung cancer in male smokers. Even though there was no significant association noticed between E-cadherin expression and the majority of clinicopathological factors analyzed, nor with the survival, we still noted a relation between tumors with reduced E-cadherin expression and the high proliferative activity, appreciated through the Ki-67 mitotic index. E-cadherin plays a major role in intercellular adhesion. The reduced expression of E-cadherin indicates an unfavorable prognosis, and can be a useful prognosis factor in NSCLC – for the patients with reduced expression of the E-cadherin/α-catenin complex requiring chemotherapy or radiotherapy.

Conflict of interests
The authors declare no conflict of interests.

Author contribution
Joana Cristina Talpoș has equal contribution and thus shares first authorship.

References


Expression of E-cadherin in lung carcinoma, other than those with small cells (NSCLC)


**Corresponding authors**

Teodora Smaranda Arghirescu, Associate Professor, MD, PhD, Discipline of Pediatrics, “Victor Babeș” University of Medicine and Pharmacy, 2 Iosif Nemoianu Street, 300011 Timișoara, Romania; Phone +40732–890 543, e-mail: sarghirescu@yahoo.com

Roxana Folescu, Senior Lecturer, MD, PhD, Discipline of Anatomy and Embryology, “Victor Babeș” University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40729–104 103, e-mail: roxanafolescu08@gmail.com

Received: January 19, 2017

Accepted: March 12, 2018