Expression of multidrug resistance-associated markers, their relation to quantitative pathologic tumour characteristics and prognosis in advanced ovarian cancer

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Abstract. Mean nuclear area has been consistently shown by different researchers to be a strong and independent prognostic factor in advanced ovarian carcinoma. However, the biological background of the prognostic value of nuclear area remains unclear. Others have found that the multidrug-resistance (MDR) related protein LRP has strong prognostic value. In the present study we have analysed whether the mean nuclear area and LRP are related in tumour tissue of the ovary obtained at the debulking operation before the administration of chemotherapy in 40 patients. The mitotic activity index, volume percentage epithelium, standard deviation of nuclear area and the other MDR-related proteins P-glycoprotein (JSB-1, MRK-16) and MRP have been investigated additionally for correlations and prognostic value.

No correlations were found between the morphometrical features and MDR-related proteins. Mean nuclear area tended to be larger in LRP positive tumours, but the correlation was not significant. In multivariate analysis LRP-protein expression and mean nuclear area had independent prognostic value.

Further studies are required to elucidate the biological background of the strong prognostic value of mean nuclear area in advanced ovarian cancer.

Keywords: Ovary, carcinoma, multi drug resistance, prognosis, morphometry

1. Introduction

Ovarian cancer remains the cancer of the female genital tract which deserves our greatest attention, since it forms the leading cause of death among all gynaecological cancers and is responsible for 6.5% of cancer deaths among women [30]. In the majority of ovarian cancer patients, disease has already spread to the peritoneal cavity or retroperitoneal lymph nodes (according to the International Federation of Gynecology and Obstetrics = FIGO stage III) or has metastasized to the abdominal wall, liver or even beyond the abdominal cavity (FIGO IV), as confirmed by diagnostic procedures and staging laparotomy. Treatment for patients with advanced ovarian cancer usually consists of cytoreductive surgery followed by cis- or carboplatin combination chemotherapy, which has numerous serious toxic side effects. In spite of these intensive treatment strategies 70% of patients will die of their disease with a median survival time of two years, be-
cause their tumours become unresponsive to combination chemotherapy [21]. Therefore, it is considered important to develop accurate prognostic factors to select those patients who are expected to obtain benefit from cis-platin based combination chemotherapy. Then, alternative treatments aimed either at palliation or improvement of survival could be offered to those patients who are not expected to benefit.

Several prognostic variables in patients with advanced ovarian cancer have been identified and the most important among these are FIGO stage and residual tumour status after cytoreductive surgery [10]. Evidence has accumulated that quantitative pathological variables like the mean nuclear area of tumour cells (MNA), mitotic indices (MI) and the volume percentage of epithelium (VPE) have important prognostic value in ovarian cancer, stronger than DNA-ploidy by flow cytometry [2,4,5,8,12–15,20,22,30]. An important advantage of these techniques is that objective and reproducible values are obtained. Obviously, they are correlated to biological processes taking place in neoplastic growth, like proliferation (MI), nuclear differentiation (MNA) and architectural differentiation (VPE).

The emergence of multidrug-resistance (MDR) in the tumour cell population plays an important role in the success of chemotherapeutical treatment in advanced ovarian cancer. In vitro resistance to platinum and alkylating agents is caused by a decrease in drug accumulation, enhanced detoxification and increased DNA repair capacity [18]. MDR to natural products is linked to the over-expression of the MDR1 gene product, P-glycoprotein (Pgp) [11] and alteration in topoisomerase II activity [6]. To date, most clinical studies in ovarian carcinoma have failed to demonstrate a role for any of these mechanisms as a major determinant of response to chemotherapy and survival [1,7,34,35]. Recently, two new drug resistance related proteins have been described, the MDR related protein (MRP) and the lung resistance protein (LRP). MRP is localized in the plasma membrane and it may cause resistance by extrusion of drugs out of the cell [33]. The LRP-gene product is localized immunohistochemically in the cytoplasm, in a granular pattern suggestive of a storage in vesicles. The LRP-gene was recently localized to the 16p13.2–16p11.2 chromosomal segment in human metaphases proximal to MRP at band 16p11.2 [29]. The LRP gene was recently cloned and sequenced in our laboratory and its product LRP was identified as the major human vault protein [26]. Vaults are cellular organelles which are thought to mediate nucleocytoplasmic transport of a wide variety of substrates [19,23]. Independent amplification of either or both the MRP and the LRP genes may be associated with the expression of acquired drug resistance [29].

In a recent study it was shown that the expression of the novel MDR-related protein LRP is a strong and independent prognostic factor to predict response to cis-platin combination chemotherapy and survival in advanced ovarian cancer [17].

Therefore, the objectives of the current investigation were to analyse whether relations exist between the expression of MDR-related proteins and quantitative pathological tumour characteristics in advanced ovarian cancer. Additionally, their value in predicting survival after cis-platin combination chemotherapy was assessed.

2. Material and methods

2.1. Patients

Forty patients diagnosed between 1984 and 1993 at the Free University Hospital, Amsterdam with advanced ovarian cancer of the common epithelial types were selected from whom frozen material from the primary tumour was available before chemotherapy. Borderline tumours were excluded. The age at diagnosis ranged from 29 to 84 years (median 68 years).

Nine patients were not treated with either cytoreductive surgery or cis-platin combination chemotherapy and were excluded from survival analysis, leaving 31 patients. Follow-up information was available in all cases and updated. The clinico-pathological characteristics and correlation of these quantitative pathological features are summarized in Tables 1 and 2.

2.2. Tissue processing and histopathology

Fresh tumour material was cut in 0.5 centimetre thick slices and fixed in neutral buffered formaldehyde. Four micron thick haematoxylin and eosin (H&E) stained sections were cut from the paraffin blocks for diagnosis and quantitative measurements. Histological types were assessed according to WHO criteria [27]. Twenty-eight were of the serous type, seven were mucinous, one was endometrioid and four were undifferentiated adenocarcinomas. One carcinoma was classified as well (grade I), nine as moderately (grade II) and thirty as poorly differentiated (grade III) [3]. The investigations for the immunohistochemical detection of MDR expression were performed on snap frozen (liquid nitrogen) material from the primary ovarian tumour.
Table 1

Correlations between clinicopathologic patient characteristics and the expression of MDR-related proteins in 40 patients with advanced ovarian cancer (tested with Pearson Chi-square statistics with Yates’ correction)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pgp expression$^1$</th>
<th>MRP expression$^2$</th>
<th>LRP expression$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>FIGO stage</td>
<td>Negative Positive</td>
<td>Negative Positive</td>
<td>Negative Positive</td>
</tr>
<tr>
<td>III ($n = 24$)</td>
<td>18 6 0.94</td>
<td>8 15 0.69</td>
<td>8 16 0.52</td>
</tr>
<tr>
<td>IV ($n = 16$)</td>
<td>13 3</td>
<td>7 8</td>
<td>3 13</td>
</tr>
<tr>
<td>Residual disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2 cm ($n = 13$)</td>
<td>7 6 0.04</td>
<td>3 10 0.25</td>
<td>4 9 1.00</td>
</tr>
<tr>
<td>&gt;2 cm ($n = 27$)</td>
<td>24 3</td>
<td>12 13</td>
<td>7 20</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I &amp; II ($n = 10$)</td>
<td>5 5 0.05</td>
<td>5 4 0.46</td>
<td>1 9 0.31</td>
</tr>
<tr>
<td>III ($n = 30$)</td>
<td>26 4</td>
<td>10 19</td>
<td>10 29</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absent ($n = 12$)</td>
<td>21 5 0.59</td>
<td>10 15 1.00</td>
<td>7 19 0.98</td>
</tr>
<tr>
<td>present ($n = 26$)</td>
<td>8 4</td>
<td>4 7</td>
<td>4 8</td>
</tr>
<tr>
<td>Histology</td>
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<td></td>
</tr>
<tr>
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<td>21 7 0.87</td>
<td>10 18 0.68</td>
<td>9 19 0.54</td>
</tr>
<tr>
<td>other ($n = 12$)</td>
<td>10 2</td>
<td>5 5</td>
<td>2 10</td>
</tr>
<tr>
<td>Performance status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>good ($n = 17$)</td>
<td>12 5 0.84</td>
<td>5 11 1.00</td>
<td>4 13 0.83</td>
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<tr>
<td>bad ($n = 15$)</td>
<td>12 3</td>
<td>5 9</td>
<td>5 10</td>
</tr>
<tr>
<td>Age (years)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>#68 ($n = 22$)</td>
<td>15 7 0.24</td>
<td>8 13 1.00</td>
<td>3 19 0.07</td>
</tr>
<tr>
<td>&gt;68 ($n = 18$)</td>
<td>16 2</td>
<td>7 10</td>
<td>8 10</td>
</tr>
</tbody>
</table>

$^1$Pgp is p-glycoprotein, $^2$MRP is MDR related protein, $^3$LRP is lung resistance protein.

2.3. Quantitative methods

In the most representative primary tumour section from the ovary, the subjectively most poorly differentiated and epithelium rich area was marked for measurements in each case. Morphometrical and stereological assessments were done as described before [5, 8]. Briefly, the mitotic activity index (MAI) was assessed by counting the total number of mitoses in 25 fields at $\times 400$ magnification (field diameter, 450 $\mu$m). Volume percentage of epithelium (VPE) was assessed by stereological point counting, and mean (MNA) and standard deviation of the nuclear area (SDNA) of 100 systematically at random selected tumour cell nuclei were measured with an interactive morphometric and stereological video overlay system (QPRODIT, Leica, Cambridge, UK).

2.4. Monoclonal antibodies (MAbs) and Immunohistochemistry

For the study of Pgp expression two murine MAbs were used which are specific for the MDR-1 gene product, MRK-16 (IgG2) and JSB-1 (IgG1). MRK-16 recognizes an external epitope of Pgp and JSB-1 reacts against and internal epitope of Pgp [16,24,31]. For the immunohistochemical detection of MRP the rat MAb MRPr1 (IgG2a) that was developed in our laboratory was used [9]. MRPr1 has been raised after immunisation with a fusion protein containing a segment of 168 amino acids in the amino-proximal half of the MRP protein, and has been well characterized by protein blot analysis, immunocytochemical and immunohistochemical studies. MRPr1 does not cross-react with the human Pgp. For the study of LRP expression the murine MAb LRP-56 (IgG2b) was used which has been developed in our laboratory after immunization with the non-Pgp MDR human lung cancer cell line SW-1573/2R120 [29]. LRP-56 specifically recognizes a 110 kD protein overexpressed in a number of non-Pgp MDR cancer cell lines of different histogenetic origin. Immunostaining was performed on the acetone-fixed cryostat sections as described in detail elsewhere [17]. Immunohistochemical expression of the MAbs was agreed upon and scored by two observers.
without knowledge of the clinical information of the patients. Samples were scored as positive if more than 10% of the tumour cells stained (for Pgp with both MRK-16 and JSB-1). In selected cases with equivocal results, the staining was repeated until a conclusive staining pattern was arrived at.

2.5. Statistics

Descriptive statistics were calculated for all variables. Relations between MDR expression and clinicopathological variables were tested with Pearson's chi-square statistics using the Yates' correction. Differences between MDR expression (categorical variables) and quantitative pathological variables (continuous variables) were analyzed using the Mann–Whitney test. Survival and progression-free survival analysis were performed with the Mantel–Cox test and illustrated by Kaplan–Meier plots. For survival time, the time from diagnosis to death of disease or last date of follow up was used. Variables were analyzed for their prognostic value using (1) the median value, (2) in

Table 2

<table>
<thead>
<tr>
<th>FIGO</th>
<th>n</th>
<th>Survival (%)</th>
<th>Median time (mo)</th>
<th>Mantel–Cox p-value</th>
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<td>45</td>
<td>30</td>
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</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>27</td>
<td>8</td>
<td></td>
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<tr>
<td>Residual disease</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>12</td>
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<tr>
<td>&gt;2 cm</td>
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<td>Grade</td>
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<td></td>
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<tr>
<td>I &amp; II</td>
<td>7</td>
<td>14</td>
<td>8</td>
<td>5.1</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>46</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>10</td>
<td>60</td>
<td>42</td>
<td>4.1</td>
</tr>
<tr>
<td>present</td>
<td>20</td>
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<td>15</td>
<td></td>
</tr>
<tr>
<td>Pgp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#10%</td>
<td>22</td>
<td>41</td>
<td>20</td>
<td>0.6</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>9</td>
<td>33</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>MRP</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>#10%</td>
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<td>36</td>
<td>15</td>
<td>1.8</td>
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<td>&gt;10%</td>
<td>19</td>
<td>42</td>
<td>27</td>
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</tr>
<tr>
<td>LRP</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>#10%</td>
<td>7</td>
<td>71</td>
<td>N.R. a</td>
<td>5.6</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>24</td>
<td>29</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Mean nuclear area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#90 ( \mu m^2 )</td>
<td>26</td>
<td>42</td>
<td>27</td>
<td>4.5</td>
</tr>
<tr>
<td>&gt;90 ( \mu m^2 )</td>
<td>5</td>
<td>20</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>SD of nuclear area</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#14</td>
<td>10</td>
<td>60</td>
<td>N.R. a</td>
<td>6.2</td>
</tr>
<tr>
<td>&gt;14</td>
<td>21</td>
<td>29</td>
<td>12</td>
<td></td>
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<tr>
<td>Volume % epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#75</td>
<td>17</td>
<td>41</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt;75</td>
<td>14</td>
<td>35</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Mitotic activity index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#12</td>
<td>16</td>
<td>44</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt;42</td>
<td>15</td>
<td>33</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Pgp is p-glycoprotein, MRP is MDR related protein, LRP is lung resistance protein.

a N.R., not reached.
three groups of equal size, and (3) according to pre-
viously established thresholds. The thresholds result-
ing in the lowest p-values are shown. Additional prog-
nostic value of features was assessed with the multi-
variate Cox regression model (enter and remove lim-
its 0.1). Variables that yielded prognostic value in uni-
ivariate analysis were used as covariates. Hypotheses 
were evaluated at a significance level of 0.05. All sta-
tistical analyses were conducted with the Biomedical 
statistical software package (BMDP Statistical Soft-
ware, Inc., Los Angeles, CA, USA).

3. Results

The correlations between the expression of MDR 
related proteins and clinicopathological features are 
summarized in Table 1. Nine out of 40 patients (23%) 
were Pgp positive, 23/38 patients (61%) were MRP 
positive and 29/40 patients (73%) were LRP positive. 
Pgp expression was significantly correlated with resid-
ual disease status and grade, respectively. As to LRP 
expression and age, LRP positive tumours tended to be 
associated with age under 68 years.

The MAI tended to be lower in LRP negative tu-
mours. An interesting finding was that none of the 
LRP negative tumours had a MNA larger than 90 \( \mu m^2 \). 
However, no significant differences were observed be-
tween expression of the MDR-related proteins and 
quantitative pathological variables.

Twelve (39%) of the 31 evaluable patients survived 
with a median survival time of 34 months (range, 7 to 
111 months) and 19 patients died with a median sur-
vival time of 10 months (range, 2 to 30 months). FIGO 
stage, SDNA, LRP-expression, MNA and the presence 
or absence of ascites had prognostic value in order of 
decreasing sequence (Table 2).

In multivariate survival analysis, LRP expression 
and MNA had additional prognostic value. LRP-ex-
pression was selected first followed by the MNA. The 
resulting formula was: \((1.0226 \times \text{LRP-expression}) + 
(0.0273 \times \text{MNA})\) (LRP-negative = 1, LRP-positive 
= 2). When the median value of this score was used 
as the cut-off point (4.90) a favourable subgroup could 
be distinguished with 50% survival (median survival 
time, 42 months) and an unfavourable group in which 
27% survived (median survival time, 12 months) (MC 
= 6.1, \( p = 0.01 \)).

4. Discussion

In this study our principle objectives were to in-
vestigate whether or not correlations exist between 
quantitative pathological variables and the expression 
of MDR-related proteins and to assess the additional 
prognostic value of these variables in advanced ovarian 
cancer.

No significant differences in the size of nuclei 
(MNA), proliferation (MAI) or the relative amount of 
tumour epithelium (VPE) could be found in tumours 
with and without Pgp, MRP and LRP expression. The 
lack of association between Pgp and proliferative activ-
ity is in agreement with a recent study in colon cancer 
in which the proliferative activity was assessed with 
DNA flow cytometry [28]. An interesting finding was 
that in the LRP negative tumours the MNA was never 
larger than 90 \( \mu m^2 \). MNA above 90 \( \mu m^2 \) is known to 
be a very poor prognostic sign in ovarian cancer [5, 
8,20]. However, the final clue as to why patients with 
large nuclei in advanced ovarian cancer fare signifi-
cantly worse than those with smaller nuclei does not 
seem to be related to MDR protein expression, even 
more since LRP-expression and the MNA had inde-
pendent prognostic value in survival analysis. Also for 
the VPE and MAI no relations with the MDR-related 
proteins were established in this investigation. There-
fore, the biological background for the strong prognos-
tic value of mean and standard deviation of the nuclear 
area still remains to be determined.

Contrary to Pgp, MRP and LRP were frequently 
expressed in the untreated advanced ovarian cancers, 
denoting their potential as markers of clinical drug-
resistance in this tumour.

Patients presenting with FIGO stage IV, large tu-
mour cell nuclei (MNA values), ascites present, ex-
pressing LRP and a high SDNA had a very poor prog-
nosis. Histological grade was also prognostically sig-
ificant but its meaning is controversial since patients 
with poorly differentiated tumour had a better survival 
than patients with well or moderately differentiated tu-
mours. This may be explained by the fact that only one 
patient presented with a well differentiated tumour and 
the other six patients probably belonged to the poor 
prognostic side of the spectrum of moderate differenti-
atation. In the present investigation and also in previous 
studies [5,8,20,30], the nuclear area associated features 
were after FIGO stage the most important prognostic 
features for survival. These nuclear size and variation 
features may reflect intratumour heterogeneity in the 
size of tumour cell nuclei and may therefore be indica-
Correlations between quantitative pathologic variables and expression of MDR-related proteins in 40 patients with advanced ovarian cancer (Mann–Whitney test)

<table>
<thead>
<tr>
<th>MDR-expression</th>
<th>n</th>
<th>MNA median</th>
<th>p-value</th>
<th>SDNA median</th>
<th>p-value</th>
<th>VPE median</th>
<th>p-value</th>
<th>MAI median</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Pgp #10%</td>
<td>31</td>
<td>78</td>
<td>0.76</td>
<td>17.1</td>
<td>0.58</td>
<td>79</td>
<td>0.19</td>
<td>64</td>
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<tr>
<td>&gt;10%</td>
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<td>77</td>
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<td>17.6</td>
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<td>75</td>
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<td>73</td>
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<tr>
<td>MRP #10%</td>
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<td>77</td>
<td>0.74</td>
<td>17.5</td>
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<td>0.94</td>
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<td>0.79</td>
</tr>
<tr>
<td>&gt;10%</td>
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<td>77</td>
<td></td>
<td>16.5</td>
<td></td>
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<tr>
<td>LRP #10%</td>
<td>11</td>
<td>72</td>
<td>0.35</td>
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<td></td>
<td>17.5</td>
<td></td>
<td>75</td>
<td></td>
<td>42</td>
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</tr>
</tbody>
</table>

MNA is mean nuclear area, SDNA is standard deviation of nuclear area, VPE is volume percentage epithelium, MAI is mitotic activity index, Pgp is p-glycoprotein, MRP is MDR related protein, LRP is lung resistance protein.

References


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