Abstract. Aim: The purpose of the present study was to evaluate the correlation between repair cross-complementing group-1 (ERCC1) status and the outcome of platin-based chemoradiation of locally advanced cervical cancer. Patients and Methods: Tumor specimens from 112 patients with locally advanced cervical cancer were evaluated for ERCC1 expression. The outcome of these patients was retrospectively assessed in correlation with ERCC1 expression. Results: Increased expression of ERCC1 correlates with a better prognosis of cervical cancer. The 2-year overall survival was 68.6% in the group with low H-score for ERCC-1 and gradually increased in the intermediate and high H-score groups to 71.7% and 90.7%, respectively. Conclusion: The present study did not confirm the correlation of low levels of ERCC1 expression with unfavorable outcomes of patients with locally advanced cervical cancer treated with platin-based radiochemotherapy.

Patients with locally advanced cervical cancer (stages IB2–IVA) (1) are at high risk for recurrence and account for the majority of cervical cancer deaths. Since the release of the National Cancer Institute (NCI) clinical alert in 1999, the standard-of-care for locally advanced cervical cancer has involved concomitant cisplatin-based chemoradiation (2). The underlying molecular mechanism of the synergy has not yet been identified. Both Deoxyribonucleic acid (DNA) damage and repair processes are likely involved. Hydrated electrons can induce both single- and double-strand breaks in platinated DNA, but not in unmodified DNA. In addition, cisplatin modification is clearly an extremely efficient means of increasing the formation of both single- and double-strand breaks by the hydrated electrons and hydroxyl radicals created by ionizing radiation (3). For chemotherapy is only, the main cytotoxic activity of cisplatin is based on the formation of DNA adducts, which cause inter- and intrastrand cross-linking (4).

Excision repair cross-complementation group 1 (ERCC1) enzyme plays a rate-limiting role in the nucleotide excision repair pathway which recognizes and removes cisplatin-induced DNA adducts (5). ERCC1 is also an important factor in the repair of DNA interstrand crosslinks, as well as in recombination processes (6). A correlation between increased ERCC1 expression and resistance to cisplatin, and to poor survival rates has been reported for several tumor types, including squamous cell carcinoma of the head and neck (7, 8). Park et al. proposed that patients expressing low levels of ERCC1 derive the most benefit from cisplatin-based neoadjuvant chemotherapy (9).

In vitro studies showed that pre-treatment ERCC1-messenger ribonucleic acid (mRNA) mRNA levels were significantly correlated with cisplatin resistance in cervical cancer cell lines (10). For primary chemoradiation, Doll et al. showed that ERCC1 status appears to have prognostic impact on univariate analysis in patients with cervical cancer, but ERCC1 status was not independently associated with outcome on multivariate analysis (11).

In this retrospective study, we evaluated the association between ERCC1 protein expression (presented as a semi-quantitative H-score of both staining intensity and the percentage of positively stained tumor cells), progression-free survival (PFS) and overall survival (OS) in patients with locally advanced cervical cancer who were treated with simultaneous cisplatin-based chemoradiation, with or without hysterectomy.
Materials and Methods

A total of 217 patients diagnosed with locally advanced cervical cancer between 2006 and 2012 at the Charité University Hospital, Charité Mitte, Virchow, and Benjamin Franklin campuses were identified depending on retrospective databases. Seventy-two patients were excluded due to lack of documentation, incomplete radiochemistry, and prior definitive treatment for cervical cancer or active malignancy at another abdominal site.

In 33 cases out of the remaining 145, the analyses for ERCC1 could not be completed. This was either due to insufficient pathological specimen or when the primary pathological study was performed outside our campuses. The final collective of this study consisted of 112 patients.

Standard staging procedures were clinical examination with or without cystoscopy and sigmoidoscopy. Nodal status was determined by pre-treatment staging laparoscopy with systematic transperitoneal pelvic and para-aortic lymph node dissection as described elsewhere (12).

Radiochemotherapy planning. All patients underwent planning computed tomography (CT) scan (CT scanner LightSpeed; GE Healthcare, Little Chaflont, Buckinghamshire, United Kingdom) with intravenous contrast media (Xenetix 350®; Guerbet GmbH, Sulzbach, Hessen, Germany) at a slice thickness of 3.75 mm. In cases of para-aortic involvement, CT scans were carried out from the diaphragm to the lesser trochanter. In histologically-proven negative para-aortic lymph nodes (LN), the CT scan was performed from the second lumbar vertebra to the trochanter minor. The planning CT was performed while the patient was in the supine position using a knee and foot positioning device, and patients were asked to have a full bladder.

Target volumes and organs at risk (OAR). Target volumes and OAR were delineated in all axial CT slices according to the recommendations of the Radiation Therapy Oncology Group (RTOG) (13) and the International Commission on Radiation Units and Measurements Reports 50 (14). The planned target volumes (PTV) were defined on the basis of the full-bladder CT scan. They were divided into PTV-A and PTV-B (boost) volumes, and the concept of simultaneously-integrated boost (SIB) was applied. The clinical target volume (CTV-A) was defined as the macroscopic tumor, including the cervix and the corpus uteri and external, internal, common iliac, and para-sacral LNs plus/minus the para-aortic LNs with a 5-mm margin. In patients treated before 2009, a sequential boost to the parametric regions was given with 1.8 Gy single doses to 59.4 Gy total dose to the PTV-B, while PTV-A was treated as described below.

The following OARs were delineated: spinal cord, femoral heads, kidneys, bladder, rectum up to the sigmoidal loop, and the small bowel (SB) as the whole peritoneal cavity except for LNs, muscles, and OAR other than the SB. The delineation of the SB exceeded the upper and lower border of the PTV-A by two slices. Institutional dose constraints were used: SB: V45 <20%; V20 <40%; D mean <30 Gy; rectum: V40 <70%; V50 <50%; bladder: V30 <60%; V50 <30%; femoral heads: D mean <40 Gy. No constraints were used for the bone marrow.

In addition to external-beam radiation, patients underwent Ir-192-High dose rate (HDR)-brachytherapy (Gammamed 12i and Brachyvision; Varian Medical Systems, Palo Alto, CA, USA) with a total dose of 25-30 Gy (five fractions at 5-6 Gy each) delivered to the cervix including the macroscopic tumor. Since 2007, PTV for brachytherapy has been defined on the basis of the magnetic resonance imaging (MRI).

Dose prescription and planning parameters. The total dose prescribed for the PTV-A was 50.4 Gy delivered in 28 fractions (five weekly) as single doses of 1.8 Gy each. The corresponding dose prescribed to the PTV-B was 59.36 Gy delivered as single doses of 2.12 Gy each for the SIB concept and 1.8 Gy single dose to a total dose of 50.4 Gy (whole pelvis ± para-aortic LN) and 59.4 Gy to the parametrical region.

Radiation technique. Until 2007, 3D planning was performed; later patients were treated either with conventional 7-field intensity-modulated radiotherapy (IMRT) or helical tomotherapy® (HT) or Rapid Arc®.

Contouring for both IMRT modalities and HT was performed with the Therapy Planning System (TPS) of Eclipse (Varian Medical Systems). The CT datasets with contoured structures were then transferred to the Tomotherapy TPS (TomoTherapy Inc., Madison, WI, USA), enabling inverse treatment planning for photon irradiation at 6 MV with HT.

Conventional linac-based IMRT plans were calculated for photon irradiation at 6 MV using the Eclipse TPS; before 2009, 6-20 MV X-rays were used.

Treatment. Primary treatment consisted of external-beam radiation and simultaneous chemotherapy with cisplatin 40 mg/m² body surface once weekly for five applications. In case of contraindications, carboplatin was used [Area under the curve (AUC) 1.5 weekly] in only three patients (2.7% of cases). Mean treatment time was 45 days. Therapy was completed in 110 patients of our final study group (98.2%).

Follow-up. According to German Guidelines, follow-up examination was carried out four times in the first two years after treatment, then twice a year, and included a gynecological examination, detailed patient history and renal ultrasound scan. Additionally to this standard procedure, all patients underwent at least one curettage three months after completion of chemoradiation to confirm histological response.

Immunohistochemistry for ERCC1. Tumor specimens from 112 patients were evaluated for ERCC1 expression. Monoclonal antibody against ERCC1 (clone 8F1; dilution 1:50; Abcam, Cambridge, UK) was used to detect ERCC1 expression. ERCC1 staining was performed on a Ventana BENCHMARK® XT Instrument using UltraView DAB kit (Ventana Medical Systems, Tucson, AZ, USA). Briefly, for epitope retrieval, slides were exposed to heat with EDTA, then endogenous peroxidase activity was blocked by incubation with 3% H₂O₂ (ERCC1: 30 min EDTA and 4 min H₂O₂). Incubation with primary antibody to ERCC1 was carried out for 32 min at 37°C. Immunoreaction was revealed by incubation with secondary antibody for 8 min, with 3'3'-diaminobenzidine as the chromogen and Mayer’s hematoxylin as the counterstain (Figure 1).

Microscopical analyses. An experienced investigator who was unaware of clinical data evaluated ERCC1 staining under a light microscope. She recorded the percentage of the tumor cells...
expressing ERCC1. Nuclear immunoreactivity was scored as positive for ERCC1.

The staining intensity was graded on a scale of 0-3: 0, negative staining; 1, mild staining; 2, moderate staining; 3, strong staining. The percentage of positively stained tumor cells was calculated for each specimen, and a proportion score was assigned (0 if 0%, 0.1 if 1-9%, 0.5 if 10-49%, and 1 if 50% or more). This proportion score was multiplied by the staining intensity of nuclei to obtain an overall score, the final semiquantitative H-score. Score 3 was assessed as high expression, score between 1.5 and 2.5 as intermediate, and score between 0 and 1 as low expression. Statistical method. Primary outcome measures were progression-free survival and overall survival. Recurrence was defined as any histologically confirmed disease recurrence. Death from disease was defined as any complications associated with cancer as well as strict disease mortality. Progression-free survival (PFS) was measured from the date of diagnosis to the date of relapse, progression, death, or last follow up. Overall survival (OS) was measured from the date of diagnosis to the date of death, or date of last follow-up. Kaplan–Meier event-free survival curves were used to estimate 2-year rates, and the log-rank (Mantel–Cox) test was used to compare the curves for statistical significance.

Results

The median age at first diagnosis of locally advanced cervical cancer was 44 years, with a range of 26 to 82 years. The diagnosis of adenocarcinoma was confirmed in 10.7% of patients, whereas 89.3% of patients had squamous cell cancer.

These tumors were well-differentiated (G1) in 3.6% of cases, moderately-differentiated (G2) in 45.5% and poorly-differentiated (G3) in 50.9%. The cases were divided according to the International Federation of Gynecology and Obstetrics (FIGO) classification as follows: 14.3% in stage IB1 (10%, 9% and 18% for the low, intermediate and high H-score, respectively), 9.8% in stage IB2 (12.5%, 4.5% and 10%), 5.35% in stage IIA (4.5%, 5% and 6%), 43.75% in stage IIB (50%, 47.5% and 38%), 1.8% in stage IIIA (2.5%, 4.5%, and 0%), 13.4% in stage IIIB (12.5%, 9% and 16%), 6.25% in stage IVA (2.5%, 9%, and 8%), and 3.6% in stage IVB (5%, 9%, 0%), whereas the staging of 1.8% cases was unknown (2.5%, 0%, and 2%). Prior to primary chemoradiation 74% of patients underwent laparoscopic staging including systematic pelvic and para-aortic lymphadenectomy and lavage of the cul-de-sac. In 26% of the patients, primary chemoradiation was performed on the basis of the clinical staging according to FIGO. On the basis of positive curretage after completing chemoradiation, extrafascial hysterectomy was performed in 11.6% of all patients.

According to the cumulative H-score for expression, the patients were classified into three groups: Low H-score (0-1): 40 patients (35.7%); intermediate H-score (1.5-2.5): 22 patients (19.6%); and high H-score (3): 50 patients (44.6%).

There was no significant differences between characteristics of the three groups including age, tumor staging, and grading. Clinical and pathological characteristics are shown in Table I.

In our study, increased expression of ERCC1 correlates with a better prognosis of cervical cancer. The 2-year OS was 68.6% in the of low H-score group [95%Confidence Interval (95%CI)=50.4-86.8%] and it increased gradually in the intermediate and high H-score groups to 71.7% (95%CI=47.2-96.2%) and 90.7% (95%CI=78.3-100%), respectively. The 2-year PFS was 49.7% (95%CI=31.7-67.6%), 33.5% (95%CI=11.1-55.9%), and 72.7% (95%CI=56.9-88.6%) for low, intermediate and high expression of ERCC1, respectively (Table II and Figure 2).

Discussion

ERCC1 plays a rate-limiting role in the nucleotide excision repair pathway, which recognizes and removes cisplatin-induced DNA adducts, as well as in recombination processes (5, 15-16). For several types of squamous cell carcinoma, increased ERCC1 levels have been associated with reduced chemosensitivity and unfavorable oncological outcome (3-5). Despite growing data on the influence of ERCC1 expression and outcome, the interaction between low ERCC1 level in terms of genetic instability and positive therapeutic response with the addition of cisplatin to radiotherapy is still unclear. The present study summarizes the data of 112 patients with locally advanced, or node-positive, cervical cancer treated uniformly with simultaneous cisplatin-based chemoradiation, on the same bases concerning indication, dose prescription, target definition and follow-up.

The highest rates of 2-year PFS and OS were associated with a high H-score, 72.7% and 90.7%, respectively. Doll et al. evaluated the association of both mRNA and protein expression of ERCC1 with clinical outcome in patients with cervical cancer treated with radiation (17). ERCC1 protein expression was measured using quantitative immunohistochemistry. From 112 patients, 99 patients had squamous cell carcinoma, and 33 patients had adenocarcinoma. Low ERCC1 mRNA expression status was associated with worse OS (p=0.046).

The analysis of the ERCC1 expression by Hasegawa et al. (18) in 36 patients with cervical adenocarcinoma showed that patients with high ERCC1 expression had a significantly worse disease-free survival than patients with low ERCC1 expression among the 25 patients who received cisplatin-based chemotherapy or chemoradiotherapy with cisplatin (p=0.002). Moreover, univariate and multivariate analyses revealed that high ERCC1 expression was an independent prognostic factor in patients receiving cisplatin-based chemotherapy or chemoradiotherapy with cisplatin.

A second study by Doll et al. included a total of 264 patients with locally advanced cervical cancer, treated with curative-intent radical chemoradiotherapy from three major
Canadian cancer Centers (19). The data demonstrated that high pre-treatment tumoral ERCC1 expression was associated with worse survival, by univariate analysis, but was not independently associated with outcome.

Strengths of our current study are the large sample size, the standardized treatments (treatments were completed in 98.2% of cases and based on cisplatin in 97.3%) and the histological confirmation of complete response. In addition, only one experienced investigator who was unaware of clinical data evaluated all ERCC1 staining under a light microscope, thus minimizing the measurement bias, and the three ERCC1 expression groups were homogeneous for tumor staging.

In conclusion, the present study did not confirm the correlation of low levels of ERCC1 expression with unfavorable outcomes of patients with locally advanced cervical cancer who were treated with platin-based radiochemotherapy. The univariate analysis shows that the high expression of ERCC1 correlates with the highest 2-year
Discrepancy of our results with other studies might be attributed to different antibodies and cut-offs utilized in immunohistochemistry, as no standardized protocols and evaluation have been defined. Additionally, studies have suggested that the chosen reagent for in situ quantification of ERCC1 may detect other molecules of similar immunoreactivity, which may explain the lack of correlation in the current study (20). Finally, more prospective studies are needed to clarify the correlation between ERCC1 expression and response to cisplatin-based radiochemotherapy in locally advanced cervical cancer, but should also generate more reliable data on the correlation of ERCC1 with other established prognostic factors (grade, size, lymph node status), as well as molecular biomarkers (human papillomavirus status, gene analyses) and non-molecular markers (interstitial tumor pressure and hypoxia).

Table I. Clinical and pathological baseline characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
<th>Staging Value</th>
<th>% of H score in each group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 (26-82)</td>
<td>IB1 N=16</td>
<td>Low 10%  Intermediate 9%  High 18%</td>
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<tr>
<td>Histological subtype Squamous cell</td>
<td>N=100 89.3% IB2 N=11 9.8%</td>
<td>Intermediate 12.5% 4.5% 10%</td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>N=12 10.7% IIA N=6 5.35%</td>
<td>High 4.5% 5% 6%</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td>G1 N=4 3.6% IIB N=49 43.75%</td>
<td>Low 50%  Intermediate 47.5% 38%</td>
<td></td>
</tr>
<tr>
<td>G2 N=51 45.5% IIIA N=2 1.8%</td>
<td>High 12.5% 9% 16%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3 N=57 50.9% IIIB N=15 13.4%</td>
<td>Low 6.25% 9% 8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation</td>
<td>Laparoscopic N=83 74% IVB N=4 5%</td>
<td>Intermediate 5% 9% 0%</td>
<td></td>
</tr>
<tr>
<td>Secondary hysterectomy after chemo-radiation</td>
<td>N=13 11.6% Unknown N=2 3.6%</td>
<td>Low 2.5% 0% 2%</td>
<td></td>
</tr>
<tr>
<td>No operation</td>
<td>N=16 14.3%</td>
<td>Low 2.5% 0% 2%</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Two-year overall and progression-free survival and log-rank (Mantel–Cox) study of the three groups of H-score for repair cross-complementing group-I (ERCC1) expression.

<table>
<thead>
<tr>
<th>H-Score</th>
<th>Overall survival</th>
<th>Progression-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 95%CI</td>
<td>p-Value</td>
</tr>
<tr>
<td>Low (0-1)</td>
<td>68.6% 50.4-86.8</td>
<td>0.053</td>
</tr>
<tr>
<td>Intermediate (1.5-2.5)</td>
<td>71.7% 47.2-96.2</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>High (3)</td>
<td>90.7% 78.3-100</td>
<td>-</td>
</tr>
</tbody>
</table>

|                        | Median 95%CI     | p-Value                    |
| Low (0-1)              | 49.7% 31.7-67.6  | **0.010**                  |
| Intermediate (1.5-2.5) | 33.5% 11.1-55.9  | **0.001**                  |
| High (3)               | 72.7% 56.9-88.6  | -                          |

PFS and OS rates. Discrepancy of our results with other studies might be attributed to different antibodies and cut-offs utilized in immunohistochemistry, as no standardized protocols and evaluation have been defined. Additionally, studies have suggested that the chosen reagent for in situ quantification of ERCC1 may detect other molecules of similar immunoreactivity, which may explain the lack of correlation in the current study (20). Finally, more prospective studies are needed to clarify the correlation between ERCC1 expression and response to cisplatin-based radiochemotherapy in locally advanced cervical cancer, but should also generate more reliable data on the correlation of ERCC1 with other established prognostic factors (grade, size, lymph node status), as well as molecular biomarkers (human papillomavirus status, gene analyses) and non-molecular markers (interstitial tumor pressure and hypoxia).

References

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