Strong Prognostic Impact of Tumor-associated Urokinase-type Plasminogen Activator in Completely Resected Adenocarcinoma of the Esophagus

Hjalmar Nekarda, Petra Schlegel, Manfred Schmitt, Monika Stark, James D. Mueller, Ulli Fink, and J. Rüdiger Siewert
Departments of Surgery [H. N., P. S., U. F., J. R. S.], Gynecology [M. Sc.], Medical Statistics and Epidemiology [M. St.], and Pathology [J. D. M.], Klinikum Rechts der Isar, Technische Universität München, D-81675 Munich, Germany

ABSTRACT
The serine protease system has been shown to play an important role in the invasive potential of a variety of tumors. To date, however, there are little data about the expression of these proteases in esophageal carcinoma. To determine the level of expression and the significance of urokinase-type plasminogen activator (uPA) and its plasminogen activator inhibitor type 1 (PAI-1) in adenocarcinoma of the esophagus, we studied 54 tumor cases and a control group of normal gastric mucosa cases with ELISA using detergent-extracted samples. uPA and PAI-1 were significantly elevated as compared to control tissue by factors of 16 and 14, respectively. Median levels of both uPA and PAI-1 showed significant correlation with tumor pT, pN, and pM categories, whereas the presence of lymphatic invasion correlated only with the uPA content and tumor grade correlated only with PAI-1 content. Using maximally selected statistics, a cutoff value was found for uPA (2.85 ng/mg protein) but not for PAI-1, which divided the study group into significantly poorer and better survival subgroups. By univariate analysis, depth of tumor invasion (pT), lymph node involvement (pN), number of involved lymph nodes, lymph node ratio, distant nodal metastases (pM1 (lym)), lymphatic invasion, and uPA showed significant correlations with patient survival. By multivariate analysis, uPA (first rank), pN, and pM (lym) were identified as independent prognostic factors, with relative risks of 8.4, 4.1, and 4.3, respectively. In a second survival analysis method, a prognostic model was developed using classification and regression trees analysis, in which a significant difference among three patient survival groups was distinguished using the variables “number of involved lymph nodes” and “uPA content.” In summary, tumor uPA content as determined by ELISA appears to be a powerful, independent prognostic factor for survival in adenocarcinoma of the esophagus.

INTRODUCTION
The incidence of adenocarcinoma of the esophagus has risen rapidly in Europe and North America over the past 20 years (1). Several previous studies, including those from our own institution (2), have validated the concept that this tumor is a clinicopathological entity distinct from both squamous cell carcinoma of the esophagus and gastric adenocarcinoma and has unique characteristics in terms of its etiology, treatment, and prognosis (3–6).

It has become increasingly evident that, in addition to conventional TNM staging, a variety of tumor biological factors may have a significant prognostic impact in a given tumor and that these are often specific for a particular tumor entity. An area of active research in this regard is the study of tumor invasion and metastatic potential, specifically regarding the proteases that tumor cells use to carry out these processes. One large and especially important family of proteases is that of the serine proteases, which includes uPA and its serpin inhibitor, PAI-1. These molecules are not only associated with tumor invasion and metastasis (7–10), but they have also been shown to be statistically independent prognostic indicators for a variety of tumors, including those of the breast (11–14), stomach (15, 16), and colon (17). Elevated levels of serine proteases have been described in esophageal carcinoma in comparison to normal mucosa (18–20), but, to date, there has been no systematic study of the clinical impact of uPA and PAI-1 expression on the prognosis of patients with esophageal adenocarcinoma.

We carried out this prospective study to see if uPA and PAI-1 expression in esophageal adenocarcinoma correlates with clinicopathological features of the tumor but, more importantly, to determine whether uPA or PAI-1 levels have a value as prognostic indicators and, if so, to determine how they compare to conventional prognostic factors for esophageal adenocarcinoma. We report, for the first time, that tumor uPA content as determined by ELISA is a powerful, independent prognostic factor for patient survival in esophageal adenocarcinoma.

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2 To whom requests for reprints should be addressed, at Chirurgische Klinik, Klinikum Rechts der Isar, Technische Universität München, Ismaninger Strasse 22, D-81675 Munich, Germany. Phone: 49-89-4140-4086; Fax: 49-89-4140-4092.

The abbreviations used are: TNM, tumor-node-metastasis; uPA, urokinase-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; UICC, Union International Contre Cancer; CI, confidence interval; CART, classification and regression trees; RR, relative risk.
PATIENTS, MATERIALS, AND METHODS

Patient Study Group. Between April 1987 and December 1991, a consecutive series of 80 patients underwent a resection of esophageal adenocarcinoma as described elsewhere (21). After exclusion of patients with preoperative chemotherapy, 54 cases remained that were included in this study. Of these 54 patients, 42 (78%) had tumors that were completely resected (UICC Ri). Four of these 42 patients died within 3 months of their resection and were excluded from survival analysis. The remaining group of 38 patients consisted of 33 males and 5 females. The five women had a mean age of 55 years (range, 51–81 years), and the men had a mean range of 61 years (range, 38–75 years). None of the patients received postoperative adjuvant therapy. The median follow-up time was 36 months (range, 10–63 months). Twenty (53%) of the patients died due to tumor recurrence, and an additional 4 patients had a suspected recurrence of their tumor during the follow-up period. The median survival time during the follow-up period was 33 months, and the estimated 5-year survival rate was 33%.

Operative Procedure. All of the 54 patients enrolled in the study underwent an esophagectomy with curative intent. An en bloc lymphadenectomy consisting of a mediastinal and celiac lymphadenectomy of gastric compartments D1 and D2 was performed as a part of each operative procedure (21, 22). Forty-four (81%) of the patients underwent a trans-hiatal radical esophagectomy and, in the 2 (4%) patients with a more proximally situated tumor, an esophagectomy from a trans-thoracic approach. In the remaining 8 (15%) patients, an esophagogastrectomy due to widespread primary tumor was performed. Three tumors (6%) were located at the tracheal bifurcation, 8 (14%) were below the bifurcation, and 43 (80%) in the gastroesophageal region [type 1 tumors according to Siwert and Stein (22)].

Staging and Histopathological Data. The operative specimens were examined in the Department of Pathology of the Technical University of Munich (Munich, Germany) according to a standard protocol, based on the guidelines of the UICC (23). The site, histological type according to the WHO (24), Laurén classifications (25), tumor grade, presence of lymphatic and vascular vessel invasion, perineural invasion, residual tumor, and TNM categories were determined for each tumor. Nine tumors (17%) belonged to stage I, 7 tumors (13%) belonged to stage IIA (pT1/pN0, n = 3; pT2/pN0, n = 4), 7 tumors (13%) belonged to stage IIB (pT3/pN0, n = 1; pT4/pN0, n = 6), 12 tumors (22%) belonged to stage III, and 19 tumors (35%) belonged to stage IV. The TNM categories and stages of the 38 patients included in the survival analysis are shown in Table 1.

Clinical Follow-Up. After resection, the patients were seen at 3-month intervals for the first year and at 6-month intervals thereafter. The following diagnostic tests were carried out at each clinic visit to determine possible tumor recurrence: (a) serum tumor marker levels, (b) upper abdominal ultrasound, (c) computed tomography scan of the thorax and abdomen, (d) bone scan, and (e) endoscopy. Approximately 40% of the patients were examined in the Oncology Outpatient Service of the Department of Surgery, Technical University of Munich. The remaining patients were examined by outside physicians according to this same protocol, and their data were collected at the Department of Surgery, Technical University of Munich.

Tissue Extraction and ELISA for uPA and PAI-1. After photographic documentation of the resection specimen, several tissue specimens ≥1 cm in size were obtained from the primary tumor in the fresh state. In addition to the tumor samples, 17 tissue samples from normally appearing fundic mucosa of the stomach were collected as a control group. All of the tissue samples were snap-frozen and stored in liquid nitrogen. The tissue extraction and ELISA procedures for uPA and PAI-1 were carried out as described previously (15, 26). Briefly, tissue samples that had been stored in liquid nitrogen were sectioned (5 μm) with a cryostat and stained with H&E to confirm the presence of a sufficient amount of tumor. Twenty to 30 sections (60 μm; total mass, 70–100 mg) were prepared from each tissue sample. After these sections of frozen tissue were pulverized, 400 μl of 1% Triton X-100 in Tris-buffered saline was added. This mixture was further disintegrated with a Braun Micro-Dismembranator (Braun-Melsungen, Melsungen, Germany) and then incubated overnight at 4°C with gentle rotation. This solution was then centrifuged at $10^3 \times g$ (1 h at 4°C). The supernatants were divided into 50-μl aliquots and stored in liquid nitrogen until use. Levels of uPA and PAI-1 were determined using commercially available ELISA kits (American Diagnostica Inc., Greenwich, CT). Details of the kits are described elsewhere (11, 14, 26). Levels of uPA and PAI-1 were expressed as ng/mg protein. The protein content of the tissue extracts was determined using the Pierce bicinchoninic acid protein assay kit (Pierce, Rockford, IL; Ref. 27).

Statistics. Statistical analysis was performed as described previously (15) using the BMDP software package (BMDP Statistical Software Inc., Los Angeles, CA). Differences in the levels of uPA, PAI-1, and total protein content of the various groups were compared using the Kruskal-Wallis and Mann-Whitney tests, which are appropriate median tests for even skewed data. For multiple comparisons, the closed test procedure was used. A difference of $P < 0.05$ was considered to be significant. For survival analysis, continuous as well as discrete esophageal cancer related covariates were included, all of which were considered to be constant over time. Both univariate and multivariate analysis using the Cox proportional hazard model (28) were used to test the influence of the various parameters on survival. For Cox regression analysis, continuous covariates were examined to determine whether the failure rate under analysis showed an exponential development due to the assumptions made in the Cox model. If not, maximally selected
log rank statistics were used to determine a “cutoff value” to recode a continuous variable as a binary one. This cutoff value will be optimal recording to separation into high and low levels of prognosis. Significance of a difference in prognosis choosing this cutoff value was examined using an adjustment for Ps, taking into account the arising multiple-test situation. The 95% CI for these cutoff values was calculated by a test-based method. Using CART, a prognosis model was calculated using all relevant covariables (29, 30). Briefly, CART uses optimal cutoff values in a recursive algorithm to receive a multiple regression model. Group-oriented curves for overall survival were calculated according to Kaplan and Meier (31). Significance of group differences was examined using the log-rank test. The RR of the various prognostic variables after discrimination into high and low, and the corresponding 95% CIs were estimated by the Cox model.

RESULTS

uPA and PAI-1 Content in Normal and Tumor Tissues.
The normal gastric epithelium (n = 16) used as control tissue had a median uPA content of 0.14 ng/mg of protein (range, 0.06–0.35) and a median PAI-1 content of 0.09 ng/mg of protein (range, 0.02–0.41). The entire group of adenocarcinomas had median values of 2.21 ng/mg of protein (range, 0.6–15.5) for uPA and 1.25 ng/mg of protein (range, 0.2–51) for PAI-1. Differences between tumor samples and normal tissues were statistically significant (P < 0.0001) for both uPA and PAI-1 (Fig. 1). There was a significant but weak correlation (r = 0.38) between tumor uPA and PAI-1. The median protein concentration of the tumor tissue extracts was 5.8 mg/ml cytosol supernatant (range, 1.24–11.5 mg/ml), which was also significantly higher than normal mucosal tissue (1.67 mg/ml; range, 0.8–5.5). uPA and PAI-1 antigen levels showed no correlation with the extent of inflammation in the investigated specimens, a feature that was evaluated on the routine H&E sections of the frozen tissues.

Correlation of uPA and PAI-1 with TNM Category.
The uPA and PAI-1 antigen content of the tumor tissue was compared to TNM categories according to the UICC (Table 2). A significant, positive correlation was found between median uPA and PAI-1 antigen levels and the depth of tumor invasion (pT1 vs. pT2, respectively). No difference in uPA or PAI-1 levels was observed between early carcinomas and carcinomas, which had infiltrated the muscularis propria (pT, versus pT2).

A statistically significant correlation was seen between uPA and PAI-1 antigen levels and nodal status, both in terms of regional lymph node involvement (pN0 versus pN1) and of involvement of the distant lymph nodes of the celiac trunk (pM0 versus pM1 lymph). The number of involved lymph nodes was also related to uPA and PAI-1 levels. For uPA there was a statistical difference between cases with no involved lymph nodes (N0) and those with one to six involved lymph nodes and, for both uPA and PAI-1, between cases with no involved lymph nodes and those with more than six involved nodes. However, no linear correlation between the number of involved lymph nodes and either uPA or PAI-1 tumor levels was present.

Correlations between tumor stage and uPA or PAI-1 were significant. Tumors with distant lymphogenous metastases (stage IV) had higher primary tumor levels of uPA and PAI-1 than did the tumors that had not metastasized or had only metastasized to regional lymph nodes (stages I, II, and III, respectively). uPA and PAI-1 levels were also elevated in tumors that had lymph node metastases but were limited to the muscularis propria (stage IIB) compared to those without lymph node metastases (stage I and IIA, respectively). Neither of these
tendencies could be shown to be significant in the closed test procedure for multiple testing. There was also a tendency for uPA and PAI-1 levels to be higher in tumors that had been incompletely resected (UICC stage R1/R2; Fig. 1).

**Correlation of uPA and PAI-1 with Histopathological Characteristics.** Tumor tissue uPA and PAI-1 antigen levels in comparison to established histopathological parameters are shown in Table 3. Among all of these various characteristics (site, size of the tumor, presence of Barrett’s epithelium, grade, WHO classification, Lauren classification, and vascular or lymphatic vessel invasion), only lymphatic vessel invasion showed a strong positive correlation to tumor uPA content which was statistically significant. The 22 cases (41%) with lymphatic vessel invasion had a median uPA level of 3.4 ng/mg of protein, in comparison to 1.6 ng/mg of protein in the negative cases. For PAI-1, there was also a difference (1.8 versus 1.6 ng/mg of protein in the negative cases). For uPA, 2.85 ng/mg of protein (95% CI, 2.73–2.89) was determined to be the optimal cutoff value for uPA. In terms of survival, this cutoff value discriminated 25 patients (66%) with uPA levels of ≥2.85 ng/mg of protein from the 13 patients (34%) with uPA levels of >2.85 ng/mg of protein (P = 0.0002). For PAI-1, a cutoff value of 1.91 ng/mg protein was determined that discriminated 26 patients (68%) with PAI-1 levels of ≥1.91 ng/mg of protein from 12 patients (32%) with PAI-1 levels of >1.91 ng/mg protein. The difference in survival was just below the level of statistical significance (P = 0.09). Actuarial survival curves (Kaplan-Meier) illustrate the increased hazard rate for patients with high tumor uPA antigen levels (Fig. 2B). The median survival time of the low-risk group of patients was significantly longer at 51 versus 12 months for the group of high-risk patients. In the low-risk group, only 8 of 25 patients (32%) died of recurrent disease, whereas in the high-risk group, only one of the original 13 patients was still alive after 4 years.

**Univariate and Multivariate Cox Analysis.** By univariate Cox analysis (Table 4), significant prognostic variables for survival were depth of tumor invasion (pT), lymph node involvement (pN), distant nodal metastases (pM (lym)), number of positive lymph nodes, the lymph node ratio, lymphatic vessel invasion of peritumoral tissue, and uPA antigen content of the tumor. Sex of the patient, associated Barrett’s mucosa, site of tumor, WHO and Lauren classification, grade, vascular vessel and perineural invasion, and PAI-1 did not show any correlation with survival.

To determine the independent value and the RR of the
Table 3  Correlation of uPA and PAI-1 content with histological parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>uPA (ng/mg protein)</th>
<th>PAI-1 (ng/mg protein)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Upper thoracic portion</td>
<td>3</td>
<td>6</td>
<td>2.7 (1.6–5.3)</td>
<td>1.1 (0.7–7.8)</td>
<td></td>
</tr>
<tr>
<td>Mid thoracic portion</td>
<td>8</td>
<td>15</td>
<td>3.3 (0.6–12)</td>
<td>3 (0.4–51)</td>
<td></td>
</tr>
<tr>
<td>Lower thoracic portion</td>
<td>43</td>
<td>80</td>
<td>1.8 (0.6–16)</td>
<td>n.s.</td>
<td>1.2 (0.2–15)</td>
</tr>
<tr>
<td>Associated Barrett’s mucosa</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>20</td>
<td>2.8 (0.9–5.4)</td>
<td>2.3 (0.7–7.8)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
<td>80</td>
<td>1.9 (0.6–16)</td>
<td>n.s.</td>
<td>1.2 (0.2–51)</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>43</td>
<td>1.9 (1.1–5.4)</td>
<td>1.1 (0.2–3.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>52</td>
<td>2.6 (0.6–16)</td>
<td>1.8 (0.7–51)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1.1 (0.6–1.6)</td>
<td>n.s.</td>
<td>0.7 (0.4–1)</td>
</tr>
<tr>
<td>Lauren classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intestinal type</td>
<td>42</td>
<td>78</td>
<td>1.9 (0.6–16)</td>
<td>1.2 (0.2–16)</td>
<td></td>
</tr>
<tr>
<td>Mixed type</td>
<td>10</td>
<td>19</td>
<td>2.6 (0.6–12)</td>
<td>1.8 (0.8–51)</td>
<td></td>
</tr>
<tr>
<td>Diffuse type</td>
<td>2</td>
<td>0.4</td>
<td>3.7 (2.1–5.3)</td>
<td>n.s.</td>
<td>3.8 (2.8–4.8)</td>
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<tr>
<td>WHO classification</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>24</td>
<td>44</td>
<td>2.1 (0.6–5.4)</td>
<td>1.2 (0.2–4)</td>
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<tr>
<td>Papillary</td>
<td>18</td>
<td>33</td>
<td>1.9 (0.6–16)</td>
<td>1.1 (0.3–16)</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
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<td>7</td>
<td>4.4 (2.9–5.3)</td>
<td>3 (1.2–8)</td>
<td></td>
</tr>
<tr>
<td>Signet ring cell</td>
<td>1</td>
<td>2</td>
<td>5.3</td>
<td></td>
<td></td>
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<tr>
<td>Undifferentiated</td>
<td>7</td>
<td>13</td>
<td>2.1 (0.6–12)</td>
<td>n.s.</td>
<td>1.2 (0.4–51)</td>
</tr>
<tr>
<td>Perineuralcarcinosis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>51</td>
<td>94</td>
<td>2.1 (0.6–16)</td>
<td>1.2 (0.2–51)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>6</td>
<td>2.8 (0.6–6.7)</td>
<td>3.4 (1–16)</td>
<td></td>
</tr>
<tr>
<td>Lymphatic vessel invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>59</td>
<td>1.6 (0.6–12)</td>
<td>1.2 (0.2–51)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>41</td>
<td>3.4 (0.6–16)</td>
<td>1.6 (0.6–16)</td>
<td></td>
</tr>
<tr>
<td>Blood vessel invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53</td>
<td>98</td>
<td>2.1 (0.6–16)</td>
<td>1.2 (0.2–51)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>2</td>
<td>5.3</td>
<td>n.s.</td>
<td>7.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Median value (range).

<sup>b</sup> Kruskall-Wallis test, P < 0.05; n.s., not significant.

<sup>c</sup> Mann-Whitney two-sample test, G<sub>G2</sub>, P = 0.02.

Prognostic factors significantly correlated with survival by univariate analysis, multivariate analysis using the Cox model was performed. The variables were the categorized variables of the TNM staging system and histopathological factors such as uPA and PAI-1 content. Three factors were found to be of independent prognostic value: regional lymph node involvement (pN), distant nodal metastasis (pM (lym)) and uPA antigen content (Table 4). For uPA, the RR of death was 8.4 times higher in the 13 (34%) high-risk patients than it was in the 25 low-risk patients. For regional lymph node involvement, RR was 4.1 for the 24 (63%) pN<sub>1</sub> patients, compared with the 14 pN<sub>0</sub> patients. The eight (21%) pM<sub>1 (lym)</sub> patients involved lymph nodes in the celiac trunk had a RR of 4.3-fold in comparison to the pM<sub>0</sub> patients.

**CART Analysis.** Because a subgroup analysis was inappropriate due to the small numbers of patients and because continuous variables (such as the number of involved lymph nodes, the ratio of involved/resected lymph nodes, age, and tumor size) in the Cox model can only contribute significant information in a categorized form using optimal cutoff values, we set up a prognosis model using CART analysis using those variables shown by univariate analysis to be significantly associated with survival. Only two variables were selected by CART analysis, the number of involved lymph nodes and the uPA antigen content of the primary tumor. A significant difference was seen in survival between the groups with no or one involved lymph node and the group with more than one lymph node involved (Fig. 3). The high-risk group with more than one involved lymph node could be further divided with respect to tumor uPA content. The cutoff value for uPA content that emerged was identical to that of the previous analysis of the entire study group, 2.85 ng/mg protein. In the group of 18 (47%) patients with the better survival probability (no or one involved lymph node), 4 (22%) patients died due to tumor recurrence. This group had a median survival time of >60 months and an estimated 5-year survival rate of 60%. The remaining 20 (53%) patients with more than one involved lymph node were divided equally into groups of 10 each on the basis of the 2.85 ng/mg of protein uPA content level. The 10 patients with a uPA level of <2.85 ng/mg protein had a survival probability of only 11 months. All of these patients died of tumor recurrence within 3 years.

**DISCUSSION**

Various tumor biological factors have been reported to be associated with the prognosis of patients with esophageal ade-
Fig. 2  Cutoff point (2.85 ng/mg of protein) for uPA (A), determined by maximally selected log-rank statistics, and the corresponding Kaplan-Meier survival curve with 95% CIs (B).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Univariate analysis (P)</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor depth (pT)</td>
<td>T1_{2/3}T1_{a}</td>
<td>0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>Node involvement (pN)</td>
<td>N_{2/3}N_{1}</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>Metastasis (pM (lym))</td>
<td>M_{2/3}M_{1}</td>
<td>0.007</td>
<td>4.3</td>
</tr>
<tr>
<td>Lymphatic vessel invasion</td>
<td>Yes/no</td>
<td>0.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>uPA (ng/mg protein)</td>
<td>≤2.85/&gt;2.85</td>
<td>0.0002</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Not significant in univariate analysis (P ≥ 0.05); grading (G_{1,2/3,4}), WHO classification (differentiated/undifferentiated), Laurén classification (intestinal/nonintestinal type), PAI-1 (cutoff value vs. 1.91 ng/mg protein), perineural invasion, site, diameter (≥/>3.5 cm), and associated Barrett mucosa. n.s., not significant.

Esophageal adenocarcinoma, including: ploidy (32, 33), transforming growth factor α (34, 35), epidermal growth factor receptor (35) c-erb-B2 (32, 36, 37), p53, (36), E-cadherin, and the catenins (38), of which only ploidy (33) and c-erb-B2 expression (32) have been shown to have an independent, statistically significant impact on prognosis.

The interplay between the serine protease uPA, its cell surface receptor uPA-R, and its inhibitor PAI-1 is increasingly recognized to be of great importance for tumor cell invasion and metastasis (39, 40). Once formed, this trimeric complex is internalized by the cell (41) and triggers an increase in cell surface-associated proteolytic activity via recycling of uPA receptor (42) and increased coexpression of the plasminogen receptor. The resulting focused cell surface proteolytic activity aids in the degradation of adjacent tumor stroma [for a review of the serine protease system and its clinical impact see Schmitt et al. (39)].

In a previous study with a smaller number of cases, Hewin et al. (20) investigated esophageal adenocarcinoma using ELISA and found uPA to be elevated by a factor of 3.5 compared to normal tissue, with PAI-1 showing no elevation. The lower protein levels he reported may be due to a lack of nonionic detergent in the tissue extraction buffer, which have been reported to increase the yield of uPA from carcinoma tissue (13). Sier et al. (19), found a 13 times higher level of uPA in the tumor tissue of squamous cell esophageal carcinoma.

Esophageal adenocarcinoma had an ~25% higher median antigen value for both uPA and PAI-1 compared to gastric adenocarcinoma, which we have studied using the same meth-
Fig. 3  A, prognostic model computed by CART analysis for the 38 completely resected esophageal adenocarcinomas. Two variables, number of involved lymph nodes and uPA content, distinguish three groups on the basis of their survival prognoses. B, Kaplan-Meier survival curves for the three groups with 95% CIs.

Details of the study and analysis are as follows:

**Fig. 3 A** shows a prognostic model computed by CART analysis for the 38 completely resected esophageal adenocarcinomas. Two variables, number of involved lymph nodes and uPA content, distinguish three groups on the basis of their survival prognoses. 

**Fig. 3 B** displays Kaplan-Meier survival curves for the three groups with 95% CIs.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Dead/Patients</th>
<th>All-time (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4/18 (22%)</td>
<td>&gt; 60 months</td>
</tr>
<tr>
<td>B</td>
<td>6/10 (60%)</td>
<td>22 months</td>
</tr>
<tr>
<td>C</td>
<td>10/10 (100%)</td>
<td>11 months</td>
</tr>
</tbody>
</table>

*ng/mg Protein

### Discussion

In several independent studies (4–6, 21, 48), it has been determined that prognostic factors for survival for patients with esophageal adenocarcinoma are: residual tumor (R classification), depth of invasion (pT), and lymph node status (pN). In collectives of completely resected esophageal adenocarcinoma (UICC R0), the only independent prognostic factors are pT category (21, 48) and lymph node status, specifically, a sudden worsening of prognosis when the percentage of positive to resected lymph nodes exceeds 30% (21).

Here, depth of invasion (pT), regional lymph node status (pN), distant lymph node involvement (pM), lymphatic vessel invasion, and uPA antigen content showed a correlation with patient survival. In multivariate analysis, uPA content, involvement of regional, and distant lymph node metastasis were the only three independent prognostic factors for patients with completely resected tumors with RRs of 8.4, 4.1, and 4.3, respectively. Thus, our study confirmed the previously identified factors but also defined uPA antigen content as a new biological prognostic factor for esophageal adenocarcinoma.

For factors shown to have a prognostic impact, CART
analysis can further identify those capable of distinguishing patient subgroups according to their prognoses (49). In our study, CART analysis found three significantly different prognostic groups that were distinguished by the two factors tumor uPA content and the number of involved lymph nodes. Interestingly, the best division into high- and low-risk groups was on the basis of involved lymph nodes with no or one involved lymph node defining the high-risk group and more than one involved lymph node defining the high-risk group, a finding that was previously reported (21). Our new finding was that the group with more than one involved lymph node can be further subdivided with respect to prognosis into statistically different survival groups using the same uPA content cutoff value previously found in the entire group.

The early reports in breast carcinoma first identified uPA alone as an independent, statistically significant prognostic factor (11, 43), but later, after a longer follow-up period, PAI-1 becomes a more powerful prognostic factor with a significance equaling or surpassing uPA (12, 14, 40). Schmitt et al. (50) recently showed that the prognostic strength of uPA and PAI-1 content changes with time, an effect that we have also observed for patients with gastric carcinoma.4 During the first 2 years after surgery, uPA has a stronger prognostic impact than PAI-1, whereas later, PAI-1 has a greater impact. The follow-up period is still too short for our collective to say if this effect also holds true for esophageal adenocarcinoma. At present, however, it appears that uPA could identify esophageal adenocarcinoma patients who will develop early tumor recurrences, thus providing a more accurate estimation of prognosis. In the future, such information could form the basis of a new therapeutic strategy through the inhibition of uPA and other such key tumor invasion molecules.

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REFERENCES


4 Unpublished observation.
Strong prognostic impact of tumor-associated urokinase-type plasminogen activator in completely resected adenocarcinoma of the esophagus.


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