The development of new treatment strategies for oesophageal cancer
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Adenocarcinomas of the gastric cardia and distal oesophagus are currently often considered as one clinical entity because of their comparably increasing incidence, prognosis and optimal treatment options. However, it is still a matter of debate whether these malignancies have the same pathogenesis and genotype.

In this study the expression of cyclooxygenase-2 (COX-2) in cardia carcinoma is correlated to clinicopathological parameters and survival and the results are compared to the prognostic value of COX-2 as described for Barrett’s carcinomas.
INTRODUCTION

Gastric cancer is a highly aggressive disease. Worldwide, it ranks second after lung cancer among malignancy associated deaths. Gastric cancer can be subdivided into distal gastric malignancies and proximal cardia carcinomas. The incidence of distal gastric carcinoma has decreased from 32/100,000 in 1935 to 8/100,000 in 1983, whereas the incidence of cardia carcinoma has increased at a rate of 5 to 10% per year over the last few decades. This increase in cardia carcinomas is comparable to the increase seen in distal oesophageal adenocarcinomas.

According to the pathological tumour node metastasis (pTNM) criteria established by the Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC), carcinoma of the gastric cardia is classified as gastric cancer, and carcinoma of the distal 8 cm of the oesophagus, including the intra-abdominal oesophagus is classified as distal oesophageal cancer. However, several studies suggest that this distinction in classification is rather artificial, since a similar phenotype was found for these malignancies. Additionally, carcinomas originating from the gastric cardia (and invading the distal oesophagus) and those originating from the distal oesophagus are mostly treated as one clinical entity, by subtotal oesophagectomy and proximal gastrectomy, with no major differences in five year overall survival and survival according to tumour stage between the two groups. Therefore, a classification in which both carcinomas are regarded as one clinical entity is nowadays often favoured.

Apart from the common pattern of increasing incidence, a comparable prognosis and the same optimal treatment options, it is questionable whether these two carcinomas have the same molecular characteristics and are one pathological entity. In a previous study, we demonstrated that the enzyme cyclooxgenase-2 (COX-2), which plays a central role in the production of prostaglandins, is upregulated in distal oesophageal adenocarcinomas developed in a Barrett’s segment. This COX-2 expression, which is in vitro associated with many oncogenetic processes (e.g. decreased apoptosis and increased proliferation, angiogenesis, invasiveness and formation of metastasis), was an independent prognostic factor for patients with a Barrett’s carcinoma. With the availability of selective COX-2 inhibitors, these findings justify the investigation of their role as a new chemopreventive or (neo-) adjuvant treatment strategy for this aggressive malignancy.

The aim of this study was to analyse the expression of COX-2 in cardia carcinomas by using immunohistochemistry and to correlate the COX-2 expression with clinicopathological parameters and survival in order to assess
whether there might be a potential place for selective COX-2 inhibition in this malignancy. In addition, these results in cardia carcinomas were compared to those previously obtained by studying Barrett's carcinomas to determine whether both upper gastrointestinal malignancies might have a comparable oncogenetic pathway.

METHODS

Patients
Between January 1st 1993 and December 31st 2000, 306 patients underwent oesophageal resection with proximal gastrectomy for adenocarcinoma of the oesophagus, gastro-oesophageal junction or gastric cardia (invading the distal oesophagus) with curative intent (i.e. locally resectable disease without distant metastases). The data from these 306 patients were prospectively collected in a database.

One hundred and fifty one patients (of which 6 patients were excluded during the immunohistochemical analyses) with a distal oesophageal adenocarcinoma developed in a histologically proven Barrett's oesophagus were analysed previously. The pathology reports of the remaining 155 patients were reviewed for the purpose of the present study. All patients were included who presented with an adenocarcinoma arising from the gastric cardia and substantially invading the distal oesophagus. The tumour was considered to be cardiac when no Barrett's metaplasia was identified and when the epicentre was in the gastric cardia, defined as the area at and immediately below the gastro-oesophageal junction (GOJ). Carcinomas with the epicentre of the mass located in the tubular oesophagus but without a Barrett's segment were excluded, to prevent inclusion of cannibalized Barrett's tumours (n=15). Tumours arising from the fundus or the corpus of the stomach and infiltrating the gastric cardia or distal oesophagus were also excluded (n=4), as were another 2 cases with an adenosquamous carcinoma. Thus, 134 patients remained for further analysis.

For all patients preoperative work-up consisted of endoscopy with histological biopsy, external ultrasonography of abdomen and neck, chest X-ray, endosonography and indirect laryngoscopy. In 98 patients (73.1%) resection was performed by a transhiatal approach without thoracotomy and extended lymph-node dissection. Thirty-six patients (26.9%) underwent oesophagectomy through a right-sided thoracotomy followed by a laparotomy in combination with two-field lymph node dissection. Patients were followed until death or July 1st 2002, ensuring a minimal potential follow-up of 18 months.
The median actual follow-up was 18 months (range 15 days to 7.8 years). They were seen at a regular basis for five years in the outpatient clinic. In the first two years patients were seen at three to four-month intervals, afterwards at six-month intervals. For the present study, patients and/or their family practitioners were contacted by phone to assess their current status when they had been discharged by the surgeon after five years. No patients were lost to follow-up. None of the patients received chemo- and/or radiotherapy preoperatively, and no adjuvant treatment was administered postoperatively. A limited number of patients received palliative external radiotherapy for symptomatic tumour recurrence. The study was done in accordance with the guidelines of the local ethics committee.

**COX-2 immunohistochemical staining**

The COX-2 immunohistochemical staining procedure is described in detail elsewhere. Briefly, formalin-fixed and paraffin-embedded specimens were sectioned (5 μm), and deparaffinized for antigen retrieval. Immunostaining was performed with a COX-2 specific mouse anti-human monoclonal antibody (160112, Cayman Chemical Co., Ann Arbor, MI, USA) in a dilution of 1:200. Every 20th sample of the trial series was a known colon adenocarcinoma specimen, in which stromal cells at an area of ulceration were scored 3+, cancer cells from 2+ to 3+, and adjacent nonneoplastic epithelium 1+ (for scoring criteria see below). Specificity of the antibody was confirmed by re-staining a randomly selected subset of specimens (every 10th sample, n=13) with and without pre-adsorption of the primary antibody with a human COX-2 control peptide (10 μg/ml, Cayman Chemical) for one hour at room temperature prior to the staining procedure (i.e. blocking controls).

COX-2 immunohistochemical staining was scored independently and in a blinded manner by two investigators (CB and AS). The following scoring criteria of the tumour cells were agreed upon before the analysis: 0, no staining; 1+, weak diffuse cytoplasmic staining (may contain stronger intensity in less than 10% of the cancer cells); 2+, moderate to strong granular cytoplasmic staining in 10-90% of the cancer cells; 3+, over 90% of the tumour cells stained with strong intensity. Scores 0 and 1 were categorized as ‘COX-2 Low’ and scores 2 and 3 as ‘COX-2 High’ for the statistical analyses (see below). The allocation of tumours to the ‘COX-2 Low’ versus the ‘COX-2 High’ category by the two investigators was similar (>90% of the specimens were categorized identically). In cases of disagreement (n=14) the slides were re-evaluated using a multiheaded microscope (CB, AS and AR). These scoring criteria and immunohistochemical control procedures are identical to those used in our previous report on COX-2 expression in oesophageal...
adenocarcinoma. In addition to tumour cell staining, positivity of the adjacent nonneoplastic mucosa, and stromal cell staining were noted.

**Statistical analysis**
The association between demographic and clinicopathological features and COX-2 expression was analysed using Student's t-test (continuous data) or Chi-squared test (categorical data). Overall survival was estimated according to the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazard model was used to evaluate various factors simultaneously. P-values of 0.05 or below were considered statistically significant. All statistical analyses were performed using the Statistical Software Package version 9.0 (SPSS INC., Chicago, IL, USA).

**RESULTS**

**Patients**
There were 113 males (84.3%) and 21 females (15.7%) with a median age of 64 years (range 39 - 83 years).

**Immunohistochemical expression of COX-2**
Normal gastric mucosa adjacent to the adenocarcinomas was consistently negative or only weakly positive for COX-2 expression. COX-2 immunoreactivity was detected in 112 of the 134 (86.7%) adenocarcinomas of the cardia, whereas 22 tumours had no COX-2 expression. Moderate to strong staining ('COX-2 High') with a granular cytoplasmic pattern was observed in 55/134 (41.0%) cases of which 2 were scored as strong and 53 as moderate. COX-2 expression was mainly localized in the neoplastic cells of the invading, peripheral margin of the tumour. Superficial tumour cells towards the lumen were often less intense, and only weak or no staining was observed in stromal cells (connective tissue cells, smooth muscle cells and blood vessels), except at sites of erosions and ulcerations and around necrosis (Figure 1).

**Correlation between COX-2 expression and clinicopathological parameters**
COX-2 expression was not significantly correlated with any clinicopathological parameter at the time of operation, although possibly a trend was seen towards a positive association with the presence of lymph node metastases (p=0.08) (Table 1A). Neither could any correlation be found between elevated COX-2 expression and the development of distant metastases or locoregional recurrences during follow-up (Table 1B).
COX-2 expression and overall survival

Kaplan-Meier curves for patient survival are depicted in Figure 2A. As can be seen in the survival curves, there is no significant difference in survival between patients in the 'COX-2 Low' category when compared to the 'COX-2 High' category with a median survival of 22 months (95% confidence interval 13 – 31) and 25 months (95% confidence interval 8 – 42), respectively (p=0.50; log-rank test). Subgroup analysis of the prognostic value of COX-2 per tumour stage did not show any significant difference in survival. Because this might be due to the low numbers of patients available per tumour stage, tumour stage was recoded into a dichotomous variable by combining categories with comparable prognosis (tumour stage Ia, Ib, II versus IIIa, IIIb, IV). There was no significant survival advantage for patients with a 'COX-2 Low' expression in either group, although there is a slight indication that COX-2 expression is a better predictor for poor outcome in relatively early cancers (p=0.17, Figure 2B).
TABLE 1A
Correlation of clinicopathological findings and COX-2 expression.

<table>
<thead>
<tr>
<th>COX-2 expression</th>
<th>Low (n=79)</th>
<th>High (n=55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient characteristics</td>
<td>(n)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>63±9</td>
<td>64±10</td>
<td>62±9</td>
</tr>
<tr>
<td>Gender</td>
<td>Male (113)</td>
<td>70 (88.6)</td>
<td>43 (78.2)</td>
</tr>
<tr>
<td></td>
<td>Female (21)</td>
<td>9 (11.4)</td>
<td>12 (21.8)</td>
</tr>
<tr>
<td>Tumour characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td>T1 (5)</td>
<td>4 (5.1)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td></td>
<td>T2 (9)</td>
<td>6 (7.6)</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td></td>
<td>T3 (112)</td>
<td>64 (81.0)</td>
<td>48 (87.3)</td>
</tr>
<tr>
<td></td>
<td>T4 (8)</td>
<td>5 (6.3)</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>N0 (20)</td>
<td>17 (21.5)</td>
<td>9 (16.4)</td>
</tr>
<tr>
<td></td>
<td>N1 (46)</td>
<td>37 (46.8)</td>
<td>27 (49.1)</td>
</tr>
<tr>
<td></td>
<td>N2 (37)</td>
<td>24 (30.4)</td>
<td>13 (23.6)</td>
</tr>
<tr>
<td></td>
<td>N3 (7)</td>
<td>1 (1.3)</td>
<td>6 (10.9)</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>M0 (128)</td>
<td>75 (94.9)</td>
<td>53 (96.4)</td>
</tr>
<tr>
<td></td>
<td>M1 (64)</td>
<td>4 (5.1)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td>Well (3)</td>
<td>2 (2.5)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td></td>
<td>Moderate (46)</td>
<td>29 (36.7)</td>
<td>17 (30.9)</td>
</tr>
<tr>
<td></td>
<td>Poor (85)</td>
<td>48 (56.4)</td>
<td>37 (67.3)</td>
</tr>
<tr>
<td>Tumour stage</td>
<td>la (3)</td>
<td>3 (3.4)</td>
<td>0 (0.0)</td>
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<tr>
<td></td>
<td>lb (3)</td>
<td>1 (1.3)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td></td>
<td>II (26)</td>
<td>16 (20.3)</td>
<td>10 (18.2)</td>
</tr>
<tr>
<td></td>
<td>Ila (57)</td>
<td>32 (40.5)</td>
<td>25 (45.5)</td>
</tr>
<tr>
<td></td>
<td>llib (39)</td>
<td>23 (29.1)</td>
<td>16 (29.1)</td>
</tr>
<tr>
<td></td>
<td>IV (6)</td>
<td>4 (5.1)</td>
<td>2 (3.6)</td>
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<tr>
<td>Operation type</td>
<td>THE (98)</td>
<td>58 (73.4)</td>
<td>40 (72.7)</td>
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<td></td>
<td>TTE (36)</td>
<td>21 (26.6)</td>
<td>15 (27.3)</td>
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<td>Radicality of resection</td>
<td>R0 (98)</td>
<td>58 (73.4)</td>
<td>40 (72.7)</td>
</tr>
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<td></td>
<td>R1 (35)</td>
<td>21 (26.6)</td>
<td>14 (25.5)</td>
</tr>
<tr>
<td></td>
<td>R2 (1)</td>
<td>0 (0.0)</td>
<td>1 (1.8)</td>
</tr>
</tbody>
</table>

1. T1: tumour limited to the submucosa. T2: tumour infiltrates muscularis propria, but not adventitia. T3: tumour infiltrates adventitia. T4: tumour infiltrates adjacent structures. 2. NO: no lymph node metastasis. N1: 1-6 lymph node metastases. N2: 7-15 lymph node metastases. N3: more than 15 lymph node metastases. 3. la: T1N0M0. lb: T1N1M0. T2N0M0. T2N1M0. T3N0M0. Ila: T2N2-3M0. T3N1M0. T4N0M0. Ilb: T2N2-3M0. T3N1M0. T4N1M0. 4. THE: transhiatal resection. TTE: transthoracic resection. 5. R0: microscopically radical. R1: microscopically nonradical. R2: macroscopically nonradical.
FIGURE 2A
Kaplan-Meier curves of 134 patients with an adenocarcinoma of the gastric cardia. There were 79 patients in 'COX-2 Low' category and 55 in 'COX-2 High' category. No significant difference was observed between the two groups (p=0.50; log-rank test).

<table>
<thead>
<tr>
<th>Survival in months</th>
<th>COX-2 Low</th>
<th>COX-2 High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>79</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>24</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>36</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>48</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>60</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

TABLE 1B
Correlation of clinical outcome parameters and COX-2 expression.

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Low (n=79)</th>
<th>High (n=55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locoregional recurrence</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>No (72)</td>
<td>41 (51.9)</td>
<td>31 (56.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>Yes (62)</td>
<td>38 (48.1)</td>
<td>24 (43.6)</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>No (76)</td>
<td>45 (57.0)</td>
<td>31 (56.4)</td>
<td>0.9</td>
</tr>
<tr>
<td>Yes (58)</td>
<td>34 (43.0)</td>
<td>24 (43.6)</td>
<td></td>
</tr>
</tbody>
</table>
Comparison with oesophageal Barrett’s carcinoma
The finding that COX-2 expression is not a prognostic variable for cardia carcinomas, is different from the results of the distal oesophageal adenocarcinomas in which it was shown an independent prognostic variable together with tumour stage and radicality of resection.\textsuperscript{12} Comparison of the COX-2 staining results showed a significantly less intense COX-2 staining in cardia carcinomas when compared to the Barrett’s carcinomas (p=0.0001, Table 2).

**TABLE 2**
Comparison of the COX-2 staining between adenocarcinomas of the gastric cardia (present study) and adenocarcinomas of the distal oesophagus developed in a Barrett’s segment (reference 12). In cardia carcinomas, significantly less (intense) COX-2 staining was found (p=0.0001).

<table>
<thead>
<tr>
<th>COX-2 expression (n)</th>
<th>Cardia carc (n=134) n (%)</th>
<th>Oesophageal carc (n=145) n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (24)</td>
<td>22 (16.4)</td>
<td>2 (1.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>1 (85)</td>
<td>57 (42.5)</td>
<td>28 (19.3)</td>
<td></td>
</tr>
<tr>
<td>2 (155)</td>
<td>53 (39.6)</td>
<td>102 (70.3)</td>
<td></td>
</tr>
<tr>
<td>3 (15)</td>
<td>2 (1.5)</td>
<td>13 (9.0)</td>
<td></td>
</tr>
</tbody>
</table>
Overall there was no significant difference in survival between the patients with a gastric cardia carcinoma and the patients with a Barrett's carcinoma (p=0.22; log-rank test), although a trend was seen towards a more favourable outcome for patients with a Barrett's carcinoma (median 25 months; 95% confidence interval 18–33 versus median 38 months; 95% confidence interval 16–60, respectively). This can at least partly be explained by a more favourable T and N stage in the patients with a Barrett's carcinoma (p<0.001).

DISCUSSION

This study shows that elevated COX-2 expression is present in the majority of patients (87%) with a cardia carcinoma. However, it did not correlate with prognosis. This finding is in contrast with our previous study in which we demonstrated that elevated COX-2 expression is an independent prognostic variable for Barrett's carcinomas, and is surprising in view of the epidemiological and clinical similarities (i.e. rapidly rising incidence, stage-by-stage prognosis and optimal surgical treatment) shared by both distal oesophageal and gastric cardia adenocarcinomas. However, it is consistent with the results of a large epidemiological study in which a substantial protective effect of nonsteroid anti-inflammatory drugs was observed for adenocarcinomas arising at distal gastric or oesophageal sites but not for cardia carcinomas.

A possible explanation for the different prognostic role of COX-2 in adenocarcinomas of the gastric cardia and distal oesophagus, is that these two diseases develop via different pathways of carcinogenesis. Apart from the significant difference in the intensity of the COX-2 expression in carcinoma cells, it was also noted that there was a discrepancy in the pattern of expression. In cardia carcinomas, the strongest (heterogeneous) COX-2 expression was seen in the invading, peripheral margin of the tumours, which was in contrast to the homogeneous and predominantly superficial, luminal expression of COX-2 seen in Barrett’s carcinomas. This suggests that in cardia carcinomas, COX-2 upregulation is a relatively late event and occurs in invasive malignant cells that are often already infiltrating the lymphatic system or blood vessels, which could explain why COX-2 expression is not a prognostic factor.

This difference in COX-2 expression with respect to intensity, localization and prognostic significance is suggestive for a different pathogenesis and different genetic constitution of these two adenocarcinomas. So far, the strongest supportive evidence that Barrett’s and cardia carcinomas represent two separate clinical entities came from an epidemiological study identifying symptomatic
reflux as a strong risk factor for oesophageal adenocarcinomas, and only a relatively weak risk factor for adenocarcinomas of the gastric cardia. Other evidence indicating that these tumours should be regarded as two different clinical entities comes from recent reports comparing intestinal metaplasia of the gastric cardia to that of the distal oesophagus. Intestinal metaplasia of the cardia is predominantly of the complete type and is associated with pathological features of the stomach, especially pangastritis. This is in contrast to the incomplete type of intestinal metaplasia in the distal oesophagus (i.e. Barrett's mucosa), which is characterized by the presence of goblet cells and non-secretory columnar cells and carries an increased risk of dysplasia and cancer. Adenocarcinomas of the cardia were also reported to have a different oncogenetic profile compared to distal oesophageal carcinomas. In particular, the prevalence of p53 mutations in cardia carcinomas is less than 50% (31-42%), whereas for oesophageal carcinomas a p53 mutation is the most frequent alteration identified (75-100%). Since p53 mutations induce COX-2 transcription in vitro by disrupting binding of p53 to the promoter region of COX-2, this could be an explanation for the less pronounced COX-2 upregulation in cardia carcinomas in comparison to Barrett's carcinomas. Another explanation for the difference in prognostic value of COX-2 for adenocarcinomas of the cardia and adenocarcinomas of the distal oesophagus arising in a Barrett's segment, might be that elevated expression of COX-2 is only a prognostic variable for poor prognosis in relatively early carcinomas. In lung cancer, upregulation of COX-2 was demonstrated to be associated with worse overall survival in patients with stage I non-small cell lung cancer. This would be in line with the prognostic significance of COX-2 expression for patients with Barrett's carcinomas who had significantly more favourable T and N stages than patients with cardia carcinomas. This is also supported by the finding that there seemed to be a trend of prognostic role for COX-2 in early carcinomas of the gastric cardia when compared to more advanced tumour stages. There are several ongoing chemoprevention and adjuvant chemotherapy trials with selective COX-2 inhibitors for gastrointestinal malignancies. Although the role of selective COX-2 inhibitors in the prevention and treatment of gastrointestinal cancer is promising, further investigations are needed before they can be incorporated in daily clinical practice. This study demonstrates that different tumours might require different treatment strategies. Therefore, it is important to gain further insight in the mechanisms by which COX-2 contributes to the carcinogenesis of various cancers. So far, the finding that COX-2 expression is not related to poorer overall survival in adenocarcinomas of the gastric cardia, makes the role of selective COX-2 inhibitors in the treatment of this cancer less promising than in Barrett's cancers.
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Submitted