Research Article

Epidermal Growth Factor Receptor Expression in Esophageal Adenocarcinoma: Relationship with Tumor Stage and Survival after Esophagectomy

Daniel Navarini,1, 2 Richard R. Gurski,1, 3, 4 Carlos Augusto Madalosso,1, 2 Lucas Aita,1 Luise Meurer,4 and Fernando Fornari2, 4

1 Programa de Pós-Graduação em Ciências Cirúrgicas, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), 90035003 Porto Alegre, RS, Brazil
2 Faculdade de Medicina, Universidade de Passo Fundo, 99010080 Passo Fundo, RS, Brazil
3 Serviço de Cirurgia Digestiva, Hospital de Clínicas de Porto Alegre, 90035003 Porto Alegre, RS, Brazil
4 Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia, Faculdade de Medicina, UFRGS, 90035003 Porto Alegre, RS, Brazil

Correspondence should be addressed to Daniel Navarini, danielnavarini@hotmail.com

Received 29 March 2012; Accepted 10 May 2012

Academic Editor: Jin-Lian Chen

Background and Aims. Esophageal adenocarcinoma (EA) is an aggressive tumor with increasing incidence in occidental countries [1–5]. Optimistic five-year survival reaches 25% in patients treated with esophagectomy [6]. Adverse biological behavior and late diagnosis explain at least in part the poor prognosis of EA [7], pointing to the need for new strategies to improve patient selection and outcome prediction.

Gastroesophageal reflux disease (GERD) is a well-known risk factor for EA, particularly in the presence of Barrett’s esophagus. This condition increases the likelihood of EA 30 times [8], with incidence of 1 new case of EA in 200 patients per year [9]. Studies also suggest a higher risk for patients with long-segment Barrett’s esophagus and a greater risk in men compared with women [1, 10]. Other established risk factors for EA include obesity and smoking [11–13].

Among prognostic tools, tumor staging using TNM system is widely employed in the management of patients with EA [14]. Staging is performed by imaging studies, but in many cases a laparoscopic or thoracoscopic intervention is necessary [15]. Prior studies have introduced biomarkers

1. Introduction

Esophageal adenocarcinoma (EA) is an aggressive tumor with increasing incidence in several countries [1–5]. Optimistic five-year survival reaches 25% in patients treated with esophagectomy [6]. Adverse biological behavior and late diagnosis explain at least in part the poor prognosis of EA [7], pointing to the need for new strategies to improve patient selection and outcome prediction.

Gastroesophageal reflux disease (GERD) is a well-known risk factor for EA, particularly in the presence of Barrett’s esophagus. This condition increases the likelihood of EA 30 times [8], with incidence of 1 new case of EA in 200 patients per year [9]. Studies also suggest a higher risk for patients with long-segment Barrett’s esophagus and a greater risk in men compared with women [1, 10]. Other established risk factors for EA include obesity and smoking [11–13].

Among prognostic tools, tumor staging using TNM system is widely employed in the management of patients with EA [14]. Staging is performed by imaging studies, but in many cases a laparoscopic or thoracoscopic intervention is necessary [15]. Prior studies have introduced biomarkers
2. Methods

2.1. Patients. In this retrospective cohort, we reviewed all cases of EA managed at Hospital de Clínicas de Porto Alegre (HCPA) between January 2000 and December 2010. Patients were selected if they met the following criteria: (1) adenocarcinoma located in the esophagus or gastro-esophageal junction (Siewert I and II); (2) treatment with transhiatal esophagectomy. Patients were excluded according to the following criteria: (1) neoadjuvant treatment with radiotherapy or chemotherapy; and (2) missing of pathology or follow-up data; (3) nonsurgical treatment; (4) Siewert III tumor. Data regarding survival were collected from medical registers or phone contact with patient’s family.

This study was conducted according to the rules of the Brazilian Ethics and approved by the Ethical Committee of the HCPA (CONEP 198984/GPPG HCPA 08-300).

2.2. Transhiatal Esophagectomy. Patients were operated following a standardized surgical approach carried out by the same surgical team. Transhiatal esophagectomy was performed as described elsewhere [22]. Briefly, patients underwent laparotomy and cervicotomía, followed by diaphragm hiatus opening and esophageal dissection with periesophageal lymphadenectomy. The esophagus was sectioned proximally in the cervical segment and distally combined with proximal gastrectomy. Alimentary transit was reconstructed with anastomosis between gastric tube and cervical esophagus.

2.3. Immunohistochemistry Analysis. Determination of EGFR expression with immunohistochemistry was carried out following a published protocol [23]. Briefly, blocks with tumor tissue were first embedded in paraffin for posterior analysis of slices stained with hematoxylin and eosin. The slices were cut in 5 μm, followed by deparaffinization and rehydration in distilled water. They underwent antigen retrieval with Proteinase K (Dako) for 5 min and washed in distilled water. Subsequently they were immersed in 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity and further washed with distilled water for 5 min. The monoclonal anti-human EGFR, clone H11 (anti-EGFR, Dako) was applied to slices at a dilution of 1:200 and incubated for 60 min, rinsed in peroxidase blocking solution (PBS) and incubated with streptavidin (1:20 dilution) by 30 min at room temperature, and washed twice with PBS for 5 min. Thereafter, chromogen diaminobenzidine was applied for 5 min, washed in common water for 3 min, and then washed in distilled water. Finally, the slices were stained with hematoxylin for 2 min, dehydrated with alcohol, and mounted for analysis.

2.4. Analysis of EGFR Expression. EGFR expression was considered positive when membrane tumor cell was stained in brown color. An external positive control was performed with placenta tissue and a cell line of esophageal squamous carcinoma with positive EGFR (Figure 1).

Tissue analysis was performed by trained investigators and reviewed by an experienced pathologist blinded to clinical and pathological patient’s information.

2.5. Statistical Analysis. Data are presented as mean ± SD, and frequencies and percentages when appropriate. The following variables were analyzed: gender, age, tumor place, tumor differentiation, surgical staging, and survival. These variables were related to EGFR expression (yes/no). Quantitative data were analyzed using t-test, whereas qualitative variables were tested with chi-square test. Survival was described using Kaplan-Meier analysis. The P value was considered statistically significant when ≤0.05.

3. Results

A total of 37 patients met the inclusion criteria for the study and had their charts reviewed. Of these, 16 patients (43.2%) had EGFR expression. The characteristics of patients grouped as positive and negative EGFR expression are shown in Table 1. Men represented the majority of patients in both groups. Tumor localization did not differ between groups, with approximately two-thirds located at the GEJ (Siewert I and II), and the remaining in the esophagus. Although well-differentiated tumors were less frequent in EGFR positive patients (44%) as opposed to 76% in EGFR negative, the difference was not statistically significant. Significant differences were found in pTNM staging. EGFR positive tumors presented higher scores either for pT (T3 + T4 = 94% versus 51%), pN involvement (94% versus 53%), or pM (57% versus 0%), in comparison with EGFR negative lesions. Accordingly tumor staging also differed between groups: all patients with positive EGFR belonged to stages III or IV, whereas most patients (62%) negative for EGFR had stage I or II lesions. EGFR expression was more frequent as higher
was the pTNM staging (I and II = 0% versus III = 47% versus IV = 100%; \( P < 0.001 \)).

Out of 37 patients, 4 died soon after the surgery due to operatory complications, including pneumonia and anastomotic leak. As presented in Figure 2, survival was significantly higher in EGFR negative patients compared to those who expressed EGFR (21.7 versus 10.5 months; \( P = 0.001 \)).

4. Discussion

Adenocarcinoma of the esophagus and gastroesophageal junction is currently considered a public health problem, given its increasing incidence and poor survival [24].

Table 1: Characteristics of patients with and without EGFR expression.

<table>
<thead>
<tr>
<th></th>
<th>EGFR + (( n = 16 ))</th>
<th>EGFR – (( n = 21 ))</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>70.4 ± 9.0</td>
<td>61.2 ± 7.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Men, ( n (%) )</td>
<td>13 (81)</td>
<td>18 (86)</td>
<td>0.716</td>
</tr>
<tr>
<td>Tumor localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophageal, ( n (%) )</td>
<td>5 (31)</td>
<td>6 (29)</td>
<td>0.999</td>
</tr>
<tr>
<td>Siewert I and II</td>
<td>11 (69)</td>
<td>15 (71)</td>
<td></td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well or moderate</td>
<td>7 (44)</td>
<td>16 (76)</td>
<td>0.086</td>
</tr>
<tr>
<td>Poor</td>
<td>9 (56)</td>
<td>5 (24)</td>
<td></td>
</tr>
<tr>
<td>pTNM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( pT1 )</td>
<td>1 (6)</td>
<td>4 (19)</td>
<td>0.036</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>6 (29)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11 (69)</td>
<td>9 (43)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 (25)</td>
<td>2 (9)</td>
<td></td>
</tr>
<tr>
<td>( pN ) negative</td>
<td>1 (6)</td>
<td>10 (47)</td>
<td>0.010</td>
</tr>
<tr>
<td>( pN ) positive</td>
<td>15 (94)</td>
<td>11 (53)</td>
<td></td>
</tr>
<tr>
<td>( pM0 )</td>
<td>7 (43)</td>
<td>21 (100)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1</td>
<td>9 (57)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tumor staging, ( n (%) )</td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>10 (48)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>7 (44)</td>
<td>8 (38)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>9 (56)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Efforts to ameliorate outcomes, including optimization of prognostic markers, can be crucial to the management of patients with this condition. Prior studies have suggested that EGFR expression might be useful in predicting outcomes in patients with EA treated with different surgical techniques [18, 19]. The purpose of the present study was to confirm the utility of EGFR expression in the prognosis of patients with this malignant condition treated with a standardized surgical approach characterized by transhiatal esophagectomy.

The main findings of our study were (1) EGFR expression was related with more advanced lesions, with higher scores for both pTNM classification and tumor staging; (2) There was a trend to the degree of tumor differentiation be poorer in cases with EGFR expression; (3) survival was significantly shorter in the group of patients who expressed EGFR. Secondary findings included a relation between EGFR positivity and older age and predominance of GEJ compromising in spite of esophageal lesions.

In the current study, EGFR expression was found in nearly half of adenocarcinomas. This is in agreement with other studies, in which EGFR expression ranges between 32% and 64% [18, 19, 25, 26]. Besides its relatively high prevalence, EGFR expression was related with more advanced lesions, with higher scores either for tumor staging, nodal involvement, or metastasis. Furthermore, lesions with expressed EGFR showed poorer tumor differentiation. These findings have been demonstrated in other studies [18, 19], indicating that EGFR expression is a marker of more advanced tumors and therefore poorer prognosis.
Survival was significantly shorter in the group of patients who expressed EGFR. This can be explained by several factors, including higher pTNM scores, poorer tumor differentiation and also older age in the group of patients with positive EGFR. These patients showed a trend in receiving more adjuvant treatment with radiochemotherapy after esophagectomy. This likely reflects advanced lesions, which usually require an aggressive approach in spite of surgical treatment [27, 28]. Prior studies have also suggested that EGFR expression is related with shorter survival [18, 19, 25, 26, 29]. It has been proposed that EGFR may participate in the carcinogenesis process of EA [30], based on the fact that EGFR may stimulate proliferation and migration of tumor cells [31, 32]. Further studies are needed to clarify this topic and assess a possible therapeutical benefit of anti-EGFR antibodies [33].

Contrasting with other studies, our patients were treated exclusively with transhiatal esophagectomy before providing tumor specimens for EGFR analysis. Thus, tissue evaluation did not suffer potential influences of other therapeutic modalities, including radiochemotherapy. In addition, EGFR analysis was carried out using immunohistochemistry, which has been considered a feasible technique for this purpose [34].

In conclusion, the current study assessed whether EGFR expression predicts tumor staging and survival in EA patients treated with transhiatal esophagectomy. We found that EGFR expression was related with older age, poor tumor differentiation, higher pTNM staging, and shorter survival as compared with EGFR negative cases. These findings confirm EGFR expression as a prognostic marker in patients with adenocarcinoma of the esophagus and GEJ treated with a standardized surgical approach. Further studies are needed to test the hypothesis that endoscopic assessment of EGFR expression can be useful in the management of EA patients.

**Funding**
This study was supported by FIPE-HCPA.

**Conflict of Interest**
The authors declare that they have no conflict of interest.

**References**


