Immunopathological Patterns of the Stomach in Adenocarcinoma of the Esophagus, Cardia, and Gastric Antrum: Gastric Profiles in Siewert Type I and II Tumors

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Background. The morphologic and immunohistochemical profiles of gastric mucosa and of the tumor were assessed in Siewert type I, type II, and gastric antrum adenocarcinomas.

Methods. Sixty-two patients, prospectively operated upon, were included in the study: 37 type II, 15 type I, and 10 antrum adenocarcinoma. Samples of the tumor, the surrounding area, and the gastric corpus and antrum were analyzed histologically, and immunostained for cytokeratins (CK) 7/20 (staining positive for cells labeled > 50%).

Results. Among the 37 type II adenocarcinomas were the following: (1) 13 of 37 (35%) had intestinal metaplasia (IM) in the stomach; (2) 24 of 37 (65%) did not show IM at any level; (3) 34 of 37 (92%) had Helicobacter Pylori (HP) infection; (4) 13 of 37 (35%) had CK7/20 expression of “Barrett’s type” (CK7/H11545/20/H11546); 24 of 37 (65%) had a “no Barrett’s type” profile (10 of 37 with CK7/H11545/CK20/H11545 and 14 of 37 with CK7+/CK20+); (5) 100% showed the same CK immunoprofile, both in IM and adenocarcinoma (measure of agreement \( k = 1, p = 0.000 \)). Type I adenocarcinomas showed the following: (1) 87.5% CK Barrett’s type, both in the tumor, and in the surrounding IM; (2) 100% gastric samples devoid of both IM and HP infection. Comparison between CK immunoprofiles in type I and type II tumors showed a difference within the two groups \( (p = 0.002) \). One hundred percent of antrum adenocarcinomas showed a no Barrett’s type CK profile, both in the tumor and in the IM of the entire stomach.

Conclusions. Data suggest that type II adenocarcinoma cannot be always considered a gastroesophageal reflux disease-related tumor; other pathogenetic pathways should be taken into consideration.

T he causes of the alarming increase in the incidence of adenocarcinoma of the esophagus and gastroesophageal junction over the past two decades is unclear [1]. Several studies have confirmed that these trends are real, excluding anatomic reclassification of adenocarcinoma of the gastric cardia as a possible confounding factor [1, 2]. As a result, the debate on possible etiologic factors is now strictly topical and it is the core of controversy that obviously extends to early diagnosis and modalities of surgical treatment for these tumors [1, 3]. Many authors do believe that adenocarcinoma of the esophagus is gastroesophageal reflux disease (GERD) related [4]. Others tend to distinguish between adenocarcinoma of the cardia, which follows an etiology similar to that of gastric adenocarcinoma [5].

Furthermore, the American Joint Commission on Cancer staging system [6] does not provide a clear anatomic definition of the cardia, although they state that the cardia belongs to the stomach. Siewert and colleagues [7] proposed a topographic classification that offers reproducible clinical nomenclature that can function as the basis for the clinical-pathological correlations needed to achieve a better understanding of esophageal and gastroesophageal junction adenocarcinomas. This classification has been adopted by many authors [8, 9]. Criteria similar to those introduced by Siewert have been adopted in our group since 1979 [10, 11] in an attempt to have a reference point in our daily work. However, these anatomic-topographic classifications have been criticized [12]. Recently, different immunohistochemical profiles of the epithelial markers, cytokeratins (CK) 7 and 20, have been...
been described for the Barrett’s related esophageal adenocarcinoma and the intestinal metaplasia (IM) and cancer in the stomach [13–16]. Those results have not been confirmed by some authors who reported a wide variability in cytokeratin expression pattern, both in tumor mass and in IM of the esophagus [17–21]. In the present study, the morphologic and immunohistochemical profile of Siewert type II adenocarcinomas has been evaluated and compared with the histologic and immunohistochemical patterns of Siewert type I tumors and adenocarcinomas of the gastric antrum. In order to possibly clarify the causes of different results reported in the literature, and in an effort to overcome some important limitations of previous studies, due to the difficulties in studying IM in peritumoral locations of advanced cancer [22], we also compared morphology and immunohistochemistry of the intact mucosa of the gastric corpus and antrum in the three types of tumors.

Material and Methods

Fifty-two patients (46 males; median age, 65.5; range, 32 to 84 years) with Siewert type I and II adenocarcinomas, consecutively operated on between January 2001 and December 2004, were included in the study. All the patients underwent a preoperative work-up, including upper gastrointestinal endoscopy (with biopsy specimens routinely performed), barium swallow, and CT scan, and were sorted according to Siewert’s classification [7]. Our group operated on type I and type II tumors with different surgical approaches [10] according to principles accepted by many [8, 9], but not all [12], surgeons.

Fifteen patients (13 males, median age, 61.8; range, 32 to 78 years) with type I adenocarcinoma in which the presence of Barrett’s mucosa surrounding the tumor was preoperatively assessed (endoscopy and histology), underwent subtotal esophagectomy and proximal gastrectomy with a laparotomy, a right thoracotomy, and a left cervical incision, with an anastomosis in the neck. The study of the stomach was completed with a multiple preoperative endoscopic mucosal biopsy protocol comprising nine different locations: three in the gastric antrum (prepyloric area, lesser and greater curvatures); one in the angulus; four in the body (two on the lesser, and two on the greater curvatures); and one on the gastric fundus.

Thirty-seven patients (33 males; median age, 66; range, 44 to 84 years) with Siewert type II adenocarcinoma underwent total gastrectomy and esophageal resection, at the level of the azygos vein, through a thoracoabdominal approach [10, 11]. Ten patients (4 males; median age, 71; range, 60 to 85 years) with adenocarcinoma of the gastric antrum were recruited as a control group. In the same period these patients were submitted to resection and were assessed by our pathologists. The local Ethics Committee approved this study and waived the need for patient consent.

Histopathologic Study of Biopsy Samples and Surgical Specimens

All surgical specimens were fixed with 10% buffered formalin. Consecutive multiple longitudinal sections 5-mm thick were taken from the entire tumor and the surrounding epithelium of the gastroesophageal junction and esophagus. In cases of total gastrectomy, multiple sections of the subcardial, fundic, and antral gastric mucosa were taken, on the lesser curvature, from the cardia to the pylorus (Fig 1). This technique has been described elsewhere [11, 23]. Sections (4 μm) were cut and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), diastase-PAS, and alcian blue-PAS technique at pH 2.5. Modified Giemsa staining was performed to search for Helicobacter Pylori (HP). In the stomach, the presence and activity of gastritis and gastric gland atrophy, and the presence of IM and HP infection, were classified according to the updated Sydney system [24]. The HP was microscopically evaluated throughout the gastric and cardiac mucosa.

The topographic distribution of IM in the stomach has been defined as follows: “focal,” if only scattered foci
The diagnosis of Barrett’s esophagus has been defined in the presence of esophageal IM (ie, columnar glandular epithelium) with mucinous “goblet” cells). Intestinal metaplasia was present in the distal esophagus (both in the lesser and the greater curvatures, from the cardia to the pylorus, also involving greater curvature of the prepyloric antrum); and “diffuse” if IM involved the entire gastric mucosa (with the exception of the fundic area) [25]. The diagnosis of Barrett’s esophagus has been defined in the presence of esophageal IM (ie, columnar glandular epithelium with mucinous “goblet” cells).

**Immunohistochemistry**

Immunohistochemistry was performed in all biopsy samples and surgical specimens. Two micrometer sections were cut, deparaffinized, and rehydrated through graded alcohol. Each section was pretreated with enzymatic digestion on a buffered solution of 0.05% protease XIV for five minutes, in a 37°C water bath. Slides were then incubated with CK20 (1:100; clone ks20.8; Dako, Carpenteria, CA) and CK7 (1:200; clone OV-TL 12/30; Biogenex, San Ramon, CA) monoclonal antibodies for one hour. Staining was revealed with a Labeled StreptAvidin Biotin (Dako) system. The reaction was developed with 3,3’-diaminobenzidine (DAB; Dako) solution. Slides were counterstained with Mayer’s hematoxylin and mounted.

Two independent pathologists (A.D. and B.C.) reviewed all cases on both histopathological and immunohistochemical grounds, and the extent of immunohistochemical staining was determined in sections containing tumor and IM of both the esophagus and stomach. A semiquantitative evaluation of CK7 and CK20 expression was performed: the immunohistochemical staining was considered positive if 50% or more of the tumor cells were stained. The percentage of cells considered positive was based on the total area of lesional tissue in 1 mm², using an optical microscope. According to Ormsby and colleagues [26] the immunohistochemical profile of CK7 and CK20 has been classified as Barrett’s type in the presence of a CK7+/CK20− profile (CK7 positivity in the superficial and deep glands, CK20 positivity in the surface epithelium), and no Barrett’s type in the other cases. Among the latter, a distinction was made between “gastric type” and “mixed type” immunoprofiles. The gastric type was diagnosed in the presence of a CK7−/CK20+ immunoprofile (CK7 absent or weakly stained in scattered tumor cells), and the mixed type in the presence of a CK7+/CK20+ immunoprofile (CK20 and CK7 strongly positive in both the superficial and deep glandular epithelium). Finally, an “absent type” was assessed in the instances of CK7 and CK20 negativity (CK20 and CK7 totally absent, both in the superficial and deep glandular epithelium) [15, 26].

**Statistical Analysis**

Tumor immunoreactivity was compared with the different histopathological subtypes by applying the χ² test, or the Fisher exact test, as appropriate. A p value of 0.05 was considered significant. Subsequently, the sensitivity, specificity, positive and negative predictive value, and diagnostic accuracy of cytokeratin staining for type I and II tumors were calculated. To validate satisfactory agreement in the evaluation of immunoreactivity between the tumors and the IM in the stomach, k-statistics were calculated. Statistical analyses were performed using a SPSS 12.00 software package (SPSS Inc, Chicago, IL).

**Results**

In Table 1 are displayed the heterogeneous CK7/CK20 immunoprofiles of Siewert type I and type II adenocarcinomas and in gastric antrum adenocarcinoma, in the function of the histopathological level of differentiation.

**Siewert Type I Adenocarcinoma**

Intestinal metaplasia was present in the distal esophagus in all cases of type I adenocarcinomas. The CK7 expres-
sion was observed in all cases of adenocarcinoma, arising on the “long segment” of Barrett’s esophagus. The CK20 expression was detected only in the signet ring cell component of the 2/15 type I adenocarcinomas. The diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of the CK7/CK20 Barrett type immunoprofile, for type I adenocarcinomas were 92%, 86.6%, 100%, 100%, and 83.3%, respectively. Histologically, all the samples taken from the stomach showed simple chronic gastritis in a quiescent phase and absence of IM and HP infection (Table 2).

### Siewert Type II Adenocarcinoma

None of type II adenocarcinomas had IM in the distal esophagus. Thirteen of 37 (35%) adenocarcinomas were of Barrett’s type (CK7+/CK20–), and 24 of 37 (65%) were of no Barrett’s type. The diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of the CK7/CK20 Barrett type immunoprofile for type II adenocarcinomas were 48.9%, 35.1%, 100%, 100%, and 29.4%, respectively. Table 2 displays the distribution of HP and IM in the stomach of the Siewert type I and type II adenocarcinomas and gastric antrum adenocarcinomas. In patients with Siewert type II adenocarcinomas, IM in the stomach was present in 13 of 37 (35%) cases, and was absent in 24 of 37 (65%) (p = 0.021). All (13 of 13) cases with IM in the stomach showed the same CK7/20 immunoprofile, both in the tumor and in IM (measure of agreement k = 1, p = 0.000).

### Gastric Antrum Adenocarcinoma

One hundred percent gastric adenocarcinomas showed a CK7/20 immunoprofile of no Barrett’s type. In 100% of cases, IM and HP were detected. The comparison between the CK immunoprofiles in Siewert type I (13 Barrett’s type, 2 no Barrett’s type) and Siewert type II (13 Barrett’s type, 24 no Barrett’s type) tumors showed a statistically significant difference within the two groups (p = 0.002).

The statistical analysis of the CK immunoprofiles in Siewert type I, Siewert type II, and gastric antrum adenocarcinomas (0 Barrett’s type, 10 no Barrett’s type) showed statistically significant differences: Siewert type I versus gastric antrum adenocarcinomas (p = 0.000), and Siewert type II versus gastric antrum adenocarcinomas (p = 0.043). The comparison between the presence of IM in the stomach that was detected in 0 of 15 of Siewert type I, in 13 of 37 (35%) of Siewert type II, and in 10 of 10 (100%) of gastric antrum adenocarcinomas, showed statistically significant differences; Siewert type I versus gastric antrum adenocarcinomas (p = 0.000) and Siewert type II versus gastric antrum adenocarcinomas (p = 0.001).

### Comment

Several studies evaluating the pathologic data of surgical specimens [9, 27] and assessing radioisotopic analysis of the lymphatic drainage [28] demonstrated that lymphatic dissemination for Siewert type II and type I adenocarcinomas were different. This issue possibly implies different surgical strategies with regard to the resection area and to the level of lymphadenectomy (subtotal esophagectomy, resection of the lower esophagus plus total gastrectomy, subtotal esophagectomy plus total gastrectomy) [7, 29]. Our experience has shown that type II tumors may present a clinical behavior closer to gastric cancer [10, 11]. The need to clarify the surgical strategy, and the discussion on the type of patients to enroll in follow-up and early diagnosis programs, have promoted research aimed to know better the biology of Siewert type I and type II adenocarcinomas.

There are numerous, heterogeneous data regarding the various cytokeratin immunoprofiles for tumors arising in the gastroesophageal junction, suggesting a different process involving Siewert type I and type II adenocarcinomas [13–15, 18–21]; however, those data are controversial. Ormsby and colleagues identified, in two studies [13, 26], a particular cytokeratin immunoprofile (CK7+/20−) typical for esophageal adenocarcinoma with areas of Barrett’s metaplasia, different from that detected in gastric adenocarcinoma. Couvelard and colleagues [14] subsequently confirmed such results in a study performed on endoscopic biopsies that reported a different...
ent immunohistochemical characterization of the areas of IM at the gastroesophageal junction; one correlated to the presence of Barrett’s esophagus developed as a result of chronic gastroesophageal reflux, another comparable with the biomorphologic characteristics typical of chronic gastritis related to HP infection. Moreover, Taniere and colleagues [15] confirmed a peculiar immunohistochemical profile for esophageal adenocarcinoma and for gastric cancer. They also identified a variable cytokeratin profile for cardia adenocarcinoma that showed intermediate immunophenotypic characteristics between the two tumors.

In more recent studies, the role of cytokeratins in characterizing tumors arising in the gastroesophageal junction is not confirmed. Flucke and colleagues [18] did not discover significant differences in cytokeratin immunoprofiles in the three types of tumors involving the gastroesophageal junction. Similar results were also reported by Gulmann and colleagues [20]. However, the retrospective design of the studies influenced the grouping criteria, and the different clones of antibodies and retrieval techniques used invalidated the reproducibility of the cytokeratin profiles. Later, Sarbia and colleagues [30], confirming the presence of a Barrett cytokeratin’s immunoprofile, did not find any diagnostic information superior to that provided by the traditional histologic methods for distinguishing the two profiles of esophageal and gastric IM. Driessen and colleagues [19] confirmed the existence of a characteristic immunohistological profile for Barrett’s mucosa, but did not find any remarkable difference in cytokeratin immunoprofiles of gastroesophageal junction tumors, and did not think that these tumors could be distinguished on this basis. More recently, the role of cytokeratin was again called into question by Van Lier and colleagues [21], who performed a study with archival biopsy material. They did not characterize the level of histologic differentiation of the tumors analyzed and that can modify the immunohistologic profiles. There are several reasons that may explain the variability in results among these groups of investigators, such as differences in the technical aspects of CK7/20 staining, observer’s agreement in interpretation of Barrett’s esophagus staining pattern, and the type of samples (biopsy versus resection’s specimen) [31]. However, the most important reason may be related to differences in sampling techniques and in the anatomic location of the biopsy’s specimens that were obtained from the gastroesophageal junction. Most problems (both endoscopic and pathologic) involve evaluating the anatomic landmarks of the gastroesophageal junction and the lack of agreement with regard to definitions and terminology [32]. Various levels of differentiation of the cancerogenic process [15], and differences in the criteria for classification of gastroesophageal junction adenocarcinoma, should also be considered. It becomes difficult to compare case series, retrospective or prospective, which are heterogeneous according to definition, classification criteria, and histopathological techniques.

In our study, to avoid or to reduce the sampling errors, we set up a prospective protocol, with rigid criteria for the morphologic and the immunohistochemical study of the tumor and the surrounding areas, for the location of the biopsy samples in the surgical specimen, and for the treatment of surgical specimens. Results from this study confirmed a peculiar and unique cytokeratin immunoprofile for type I adenocarcinoma (ie, Barrett’s adenocarcinoma). In only two of our type I tumors did we detect a “mixed” cytokeratin pattern (CK 7+/20+). We related the expression rate of CK 20 to the low grade of differentiation of the tumor, typical of “signet ring” cells tumors [15, 18]. In Siewert type I adenocarcinoma we detected a high level of diagnostic accuracy (92%), sensitivity (86.6%), and specificity (100%) for the CK Barrett’s type immunoprofile that permitted us to correctly identify this disease. In Siewert type II, a CK Barrett’s profile was recorded in 35% of cases, with a remaining non-Barrett’s profile in 65% of cases. In this group of patients, the values of the diagnostic accuracy (48.9%) and sensitivity (35.1%) of the CK Barrett’s type immunoprofile were definitely lower than those recorded in patients with Siewert type I tumors. However, the analysis of the histologic and immunohistochemical characteristics of the mucosa of the gastric corpus and antrum seems to provide new interesting elements: HP was not detected histologically in the stomach in any of type I adenocarcinomas but was present in 92% of cases with type II tumor, including all cases (13 of 37 [35%]) in which the presence of IM in the stomach was detected. A statistically strong agreement (k = 1) between the immunohistochemical profiles (4 of Barrett’s and 9 of no Barrett’s type) of type II adenocarcinoma and those detected in the areas of gastric IM associated with HP infection was also evident. Finally, it is interesting that of Siewert type II group we found the following: (1) nine had an immunoprofile in the tumor and in the IM with HP infection like those detected in gastric antrum adenocarcinoma; (2) 28 presented heterogeneous combinations of the investigated immunohistologic patterns in the tumor and in the gastric mucosa, different from the Siewert type I and gastric antrum adenocarcinoma groups.

In conclusion, our data support the theory that type II adenocarcinomas cannot be considered a unique, GERD-related, pathological entity; other pathways should be taken into consideration. Many expression-profiling studies have been conducted over the last few years to understand the biology of esophageal adenocarcinoma and to identify biomarkers that can be targeted [33, 34]. Future investigations should be pointed at identifying the molecular signatures that predict treatment and pathologic outcome [35].

References