NEOPLASTIC PROGRESSION IN BARRETT'S OESOPHAGUS

 Print: Offsetdrukkerij Ridderprint B.V., Ridderkerk

NEOPLASTIC PROGRESSION IN BARRETT'S OESOPHAGUS

MALIGNE ONTAARDING IN EEN BARRETT OESOFAGUS

Proefschrift

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. Dr. P.W.C. Akkermans, M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 20 maart 1996 om 13.45 uur.

door

Maria Bernardine Emmy Menke-Pluymers

geboren te Enschede

Promotiecommissie

Promotor	:	Prof. Dr. J. Jeekel
Co-promotor	:	Dr. H.W. Tilanus
Overige leden	:	Prof. Dr. A.M.M.J. Hagemeyer-Hausman
		Prof. Dr. Th.H. van der Kwast
		Prof. J.H.P. Wilson

The publication of this thesis was financially supported by:
Janssen-Cilag BV, Astra Pharmaceutica BV, Glaxo Wellcome BV and Byk Nederland BV.

Twee schaduwdansers
eindeloos opnieuw eenheid
zoekend op de weg.
(P. Courant, 1983)

CONTENTS

CHAPTER 1

Columnar lined (Barrett's) oesophagus and oesophageal adenocarcinoma.
A review of the literature.

1.1 History and definition

1.1.1 History and definition

In his article in 1950 'Chronic peptic ulcer of the oesophagus and oesophagitis' Norman Barrett described the occurrence of ulcers in the lower part of the oesophagus lined by columnar epithelium (1). He believed that these were gastric ulcers developing within a tubular intrathoracic extension of the stomach in patients with congenitally short oesophagi. Earlier descriptions of peptic ulcers located in the oesophagus had been given by Tileston in 1906 and Lyall in 1937 (2,3). In 1953 Allison and Johnstone first used the term 'Barrett's ulcers' to indicate the presence of an ulcer in oesophageal columnar epithelium (4). They emphasized that the organ lined by gastric mucous membrane was the oesophagus, since it had no peritoneum covering its musculature and had islands of squamous epithelium. Furthermore, they believed that the condition was acquired due to the progress of oesophagitis rather than congenital. In 1957 Barrett admitted that the tubular structure he initially described was indeed the oesophagus and published further cases of the lesion classifying it under the title 'Lower oesophagus lined with columnar epithelium' (5). This columnar mucosal lining of the distal oesophagus is commonly referred to as Barrett's mucosa. Lortat-Jacob in 1957 described the same condition as endo-brachyoesophagus, which he defined as a short oesophagus whose sole criterion of shortness was its endocavitary, i.e. its mucosal appearance (6). Endo-brachyoesophagus as synonymous with Barrett's oesophagus is frequently used in the French, German and Swiss literature.

Barrett's oesophagus is a condition in which a variable length of squamous epithelium in the distal oesophagus is replaced by columnar epithelium. Since the columnar mucosa may normally extend up to 2 or 3 cm above the lower border of the lower oesophageal sphincter, we refer the term Barrett's oesophagus to the condition representing a circumferential zone of columnar epithelium of at least 3 cm in length above the gastro-oesophageal junction (7).

1.1.2 Etiology and pathogenesis

Since the early fifties there has been discussion about the etiology of Barrett's oesophagus, resulting in the congenital and acquired theory. The congenital theory was suggested by the observation that the embryonic oesophagus is originally lined with columnar

epithelium (8). During the 18th to 20th weeks of fetal growth the columnar epithelium is replaced by squamous epithelium that gradually progresses from the middle of the oesophagus in both directions. Conversion to squamous epithelium is completed before birth, but an arrest in the developmental process could account for Barrett's oesophagus. A number of reports describe familiar occurrence of Barrett's oesophagus, suggesting a genetic origin with autosomal dominant inheritance (9-11). However, other authors suggested that the gastro-oesophageal reflux and not the Barrett's epithelium is the condition that is inherited (12,13). Although it is not possible to exclude a congenital origin in some patients, today the acquired theory is generally the more accepted explanation of the pathogenesis of Barrett's oesophagus. Allison and Johnstone first suggested that Barrett's oesophagus was an acquired condition due to severe and chronic gastro-oesophageal reflux causing oesophagitis with destruction and desquamation of squamous epithelium, resulting in its replacement by the more acid resistant columnar epithelium (4). The acquired theory was supported by several authors showing that chronic oesophagitis with destruction of the squamous epithelium may heal by migration of adjacent columnar epithelium (14,15). Further support came from clinical observations in which development of a columnar-lined oesophagus was seen after gastro-oesophageal surgery, such as oesophago-gastrostomy, oesophago-jejunostomy and Heller myotomy, and in conditions associated with gastro-oesophageal reflux disease, such as scleroderma and Zollinger-Ellison syndrome (16-19). Experimental studies with animal models showed that columnar mucosa could replace a denuded part of the oesophagus in the presence of gastro-oesophageal reflux (20,21).

Patients with Barrett's oesophagus have been shown to have a number of physiological abnormalities that might contribute to the severity of gastro-oesophageal reflux disease. Manometric study of the Barrett oesophagus reveals a reduced pressure in the lower oesophageal sphincter more frequently than normal, and therefore these patients are predisposed to reflux (22). It has been demonstrated by means of pH-monitoring in the oesophagus that patients with Barrett's oesophagus have a high reflux-index, with frequent and prolonged episodes of reflux (23). In addition, there is a reduced oesophageal acid clearance due to poor oesophageal contractility or dyscoordination of the peristalsis (22-24). The increased exposure of the oesophagus to acid may also result from an enhanced basal gastric acid secretion (25). Patients with columnar epithelial metaplasia of the oesophagus have relatively reduced sensitivity to acid (26). This can cause delay in acid clearance via the

absence of stimulation of swallowing and saliva excretion when reflux occurs, and lead to poor compliance with antireflux therapy (27). Some patients have duodenogastric reflux and, consequently, bile and pancreatic juice may be present in the stomach (28). Secondary to duodenogastric reflux there is an increased exposure of the oesophagus to an alkaline environment (29). The acidic environment in the oesophagus enhances the injurious effect of the proteolytic enzymes and bile acids in the refluxed material (30). Experimentally, bile reflux has also been shown to cause columnar transformation of the oesophagus (21,30). Other factors, less commonly, are also associated with the development of Barrett's oesophagus. Spechler et al. described the development of Barrett's mucosa 24 years after accidental lye ingestion in a patient (31). In addition, three children who underwent chemotherapy for leukemia were found to have Barrett's mucosa (32).

1.2 Detection and management

1.2.1 Epidemiology

The true prevalence of Barrett's oesophagus in the general population is unknown. In a study from the Mayo Clinic the prevalence of Barrett's oesophagus found at autopsy (376 cases per 100,000 population) in the population of Olmstead county was 16 times higher than was predicted on a population-based study of clinically diagnosed patients (23 cases per 100,000 population) (33). This indicates that many people in the community have Barrett's oesphagus that remains unrecognized. There was an increase of the incidence of Barrett's oesophagus diagnosed over the past three decades, probably because of the more frequent use of endoscopy, but over the years Barrett's oesophagus continued to be found in about one of every 100 oesophageal endoscopic examinations for all clinical causes. The incidence of newly diagnosed clinical cases of Barrett's oesophagus is 9-13% of patients with symptoms of reflux oesophagitis referred to hospital for endoscopy and 44% of patients referred to hospital with peptic stricture of the oesophagus (27,34-37). The average age at the time of diagnosis of Barrett's oesophagus ranges from 55 to 64 years (7,34,38-41). There seems to be a bimodal age distribution with age incidence peaks occurring at 0 to 15 years and from 40 to 80 years (38). However, Cameron et al. demonstrated that the prevalence of Barrett's oesophagus is increased with age to reach a plateau by the seventh decade (40). It seems that

Barrett's oesophagus occurs almost exclusively in white people (7,41). There is male predominance among patients with Barrett's oesophagus with a male to female ratio varying from 2:1 to 4:1 (7,34,39-41).

1.2.2 Clinical presentation and diagnosis

Barrett's oesophagus itself does not cause symptoms, but most of the patients have the symptoms or complications of gastro-oesophageal reflux disease (25,39). The most common symptoms of patients with Barrett's oesophagus are heartburn (67-81%), dysphagia (38-68%) and regurgitation (33-67%) (34,42,43). Complications associated with Barrett's oesophagus are ulceration (14-68%), stricture (19-61%) and bleeding (30%) (7,34,35,41-46). The majority of patients with Barrett's oesophagus (70-92%) have a hiatal hernia (7,34,35,41,42,46). The association of a columnar lined lower oesophagus and adenocarcinoma has been recognized as early as in 1950 (47). The reported prevalence rate of adenocarcinoma in patients with Barrett's oesophagus varies from 8.5 to 37.5% (34,41,43,48-50). Some other diseases associated with Barrett's oesophagus have been described. Davidson found an associated duodenal ulcer in 10% of the cases and Sontag et al. reported 19 benign and 10 malignant colon neoplasms in a series of 65 Barrett's patients (51,52).

Endoscopic examination with biopsy of the abnormal epithelium is required to establish the diagnosis of Barrett's oesophagus. Barrett's mucosa in most of the cases can easily be recognized at endoscopy by the sharp demarcation between the pale glossy squamous mucosa and velvety, salmon-pink mucosa extending to the mid-oesophagus rather than its normal location at the diaphragmatic hiatus (35,39,53). The columnar lining extends proximally up into the oesophagus in irregular, fingerlike projections or as a circumferential sheet. There may also be isolated islands of Barrett's epithelium in the distal oesophagus. As a diagnostic test for Barrett's oesophagus, endoscopy alone (without biopsy) has a specificity of approximately 90% (35,54). During endoscopy oesophagitis, oesophageal ulcers and strictures may be found. Adenocarcinoma arising from Barrett's epithelium may appear endoscopically as a polypoid lesion, an ulcerated mass, or an infiltrative friable lesion simulating a benign peptic stricture (55). However, intramucosal adenocarcinoma may be patchy in distribution and without grossly recognizable neoplastic lesions on endoscopy (56).

Therefore multiple biopsies should be obtained throughout the columnar lined tubular oesophagus (e.g. at 2 cm intervals) and specimens should also be taken of any visible lesion, no matter how insignificant they may appear endoscopically.

No radiological findings are pathognomonic for Barrett's oesophagus, but the diagnosis should be suspected when strictures or ulcerations are found in the oesophagus (27,46). Winters et al. demonstrated that the sensitivity of radiologic examination for Barrett's oesophagus was 24% and the specificity 94% (35).

Although manometric studies and 24-hour pH monitoring commonly show abnormalities in the majority of patients with Barrett's oesophagus, they are not specific for this disorder.

1.2.3 Histologic features

The columnar mucosa of the oesophagus consists of a spectrum of epithelial patterns. Three different epithelial types can be distinguished and were classified by Paull and Trier (57):

1) Specialized columnar epithelium has a villiform surface and crypts that resemble intestinal mucosa. The rudimentary or well-formed villi are lined with columnar cells and mucous-secreting goblet cells. There are no parietal or chief cells. Between the villi there are cryptlike glands lined with columnar and goblet cells, in which entero-endocrine cells and Paneth cells can be found (58). Specialized columnar epithelium represents incomplete intestinal metaplasia because it lacks intestinal absorptive cells and is functionally immature (59). Recently, it was found that specialized columnar epithelium and normal colonic mucosa share a unique epitope, suggesting a histogenetic relation between specialized Barrett's epithelium and colonic-type epithelium (60). Specialized columnar epithelium is the most common type of Barrett's epithelium and tends to progress to dysplasia and adenocarcinoma at a relatively higher frequency than other types of Barrett's epithelia (4,5,7,50,61,62).

2) Junctional type epithelium has a foveolar surface with rudimentary villiform pattern, which is as well as the underlying glandular tissue composed of mucous secreting cells. Parietal, chief, Paneth or entero-endocrine cells are absent (57,58). This type of epithelium resembles the mucosa of the gastric cardia.

3) Gastric-fundic epithelium has a foveolar or pitted surface without villi, lined by mucous-

secreting cells and a deeper glandular layer that contains chief and parietal cells. This type of epithelium resembles the mucosa of the gastric body and fundus.

Any of these epithelia, or any combination constitutes the mucosa of Barrett's oesophagus. It has been suggested that columnar cells develop from a gastrointestinal stem cell, possibly the gastric mucous neck cell. Some degree of inflammation is always present in the columnar epithelium. Takubo et al. observed that, unlike the normal oesophagus with its single muscularis mucosae layer, the oesophageal segment lined by Barrett's epithelium had a double muscularis mucosae (63). This suggests that the alterations in Barrett's oesophagus are not limited merely to the epithelial cells that line the luminal surface and the glands, but also extend to the mesenchyme.

1.2.4 Treatment

The treatment of Barrett's oesophagus is primarily that of gastro-oesophageal reflux disease and its complications (39). Therapeutic goals are to relieve pain, promote healing of the oesophageal mucosa, avoid complications and prevent recurrence (64). Since the pathogenesis of gastro-oesophageal reflux is multifactorial, effective therapy can deal with contributing factors such as gastric acid secretion, lower oesophageal sphincter pressure, or gastric motility. Asymptomatic patients with uncomplicated Barrett's oesophagus do not require therapy (39,65). Symptomatic patients should start with dietary and lifestyle modifications such as elevation of the head of the bed, weight loss, avoidance of food before bedtime and cessation of smoking (66). Pharmacologic therapy usually includes antacids, H2-receptor antagonists, prokinetic agents and in some cases proton pump inhibitors. Healing of reflux oesophagitis and treatment of the symptoms in Barrett's oesophagus often requires greater reduction in acid production than cases of oesophagitis alone or in the treatment of duodenal and peptic ulcers (67). H2-receptor antagonists like ranitidine and cimetidine reduce gastric acid secretion and can provide symptomatic relief and some reduction in the severity of oesophagitis in patients with Barrett's oesophagus (68,69). However, the healing of Barrett's ulcers is slow when H2-receptor antagonists are used and treatment has to be given in large doses over a prolonged period (27,68,70). Almost total suppression of gastric acid secretion can be obtained with a H+/K+- adenosine triphosphatase inhibitor like omeprazole or lansoprazole. Good results in the treatment of even severe oesophagitis and Barrett's ulcers

with omeprazole in a relatively short period of time have been described (70-74). In the great majority of studies in which long-term medical treatment consisted of H2-receptor antagonists, no decrease in the extension of columnar epithelium was observed, although partial regression has been described (68,69,75,76). After longterm treatment with proton pump inhibitors, regression of the linear extent of the columnar mucosa and the emergence of macroscopic squamous islands within the abnormal mucosa have been demonstrated (77).

Berenson at al. documented restoration of squamous mucosa after abolition of oesophageal columnar epithelium by argon laser photoablation in patients who were treated with omeprazole (78). An experimental study in dogs showed the same results (79). However, long-term treatment outcomes have not been defined and the technique of laser ablation may not be practical for patients with extended columnar mucosa.

Antireflux operation for Barrett's oesophagus has been advocated in patients with intractable symptoms despite medical therapy or in patients whose complications are unresponsive to conservative measures (46,49,80). The Nissen fundoplication and Belsey-Mark IV procedures are the most commonly performed antireflux operations (81). In Barrett's patients, antireflux operation leads to improvement of the lower oesophageal sphincter pressure in 73-90% of the patients and normalization of the standard acid reflux test in 62-70% of the patients (7,80). Symptomatic relief has been described in 77-92% of the Barrett's patients after antireflux surgery, even after longterm follow-up, and healing of ulcera or strictures in 88-95% of all cases (7,34,45,80,82). While antireflux surgery can relieve the symptoms of oesophagitis and is an effective therapy for some complications of gastro-oesophageal reflux disease, it is not a reliable means by which to effect regression of Barrett's mucosa. A minority of patients shows any sign of regression, and when present, this is rarely complete (7,44,49,80).

1.3 Neoplastic progression of Barrett's epithelium

Since the incidence of adenocarcinoma in Barrett's oesophagus is 30 to 125 times higher than that of the general population, Barrett's oesophagus is considered a premalignant condition (39,43,48-50,83,84). Although Barrett's oesophagus carries a risk of adenocarcinoma, the value of endoscopic screening remains unclear (50,84-87). Retrospective

and prospective studies demonstrated an incidence of adenocarcinoma in Barrett's oesophagus varying from 1 per 52 to 1 per 441 patient years of follow-up (39,43,48-50,83,84). A significant better 5-year survival after oesophagectomy for adenocarcinoma has been demonstrated for patients with Barrett's oesophagus who underwent routine endoscopic surveillance (5-year survival 62%) as compared to patients not under surveillance (5-year survival 20%), due to a higher percentage of early stage carcinomas in the former group (86). On the other hand, no significant difference could be demonstrated between the survival curves of a follow-up population of patients with Barrett's oesophagus and that of an age and sex matched group from the general population (84). Although many authors have stressed the importance of annual endoscopic screening, others stated that there are no arguments to submit all patients with Barrett's oesophagus, but only those patients at high risk of malignant degeneration (50,65,84-87). Therefore, it is necessary to define factors which indicate increased risk of malignant change for selecting patients who should be offered regular screening.

1.3.1 Histological subtype and extension of Barrett's oesophagus

It has been observed that high grade dysplasia and adenocarcinoma are usually surrounded by specialized columnar epithelium, while gastric-fundic or junctional type epithelium are rarely associated with high grade dysplasia or adenocarcinoma (7,50,61,62,83,88-90). Therefore, it is thought that patients who have specialized metaplastic epithelium are most at risk for developing an oesophageal adenocarcinoma. Ransom et al. demonstrated that specialized columnar epithelium was more common in an extended columnar lined oesophagus (91). Although oesophageal adenocarcinomas can develop in short segments of Barrett's mucosa, patients with an extensive columnar lined oesophagus seem to have a higher risk of malignancy (84,88,89,91). Iftikhar et al. pointed out that the length of columnar lined oesophagus in patients with dysplasia was significantly longer as compared with patients without dysplasia (92). Since none of the patients with dysplasia had a columnar lined segment of less than 8 cm, they regarded patients with a columnar lined oesophagus of 8 cm or more to be at particular risk. In de follow-up study of van der Veen et al. all four patients who developed adenocarcinoma had also a columnar lined segment of more than 8 cm (84).

1.3.2 Dysplasia

The metaplastic columnar epithelium has the potential to undergo morphological changes resulting in dysplasia. The main histological and cytological features of epithelial dysplasia are cellular atypia and disorganized mucosal architecture (90). In order to classify the various degrees of epithelial dysplasia in Barrett's oesophagus, two grading systems have been worked out. A grading system has been evolved for dysplasia of the gastric mucosa, based on criteria such as cellular atypia, abnormal differentiation and disorganized architecture of the mucosa, which classifies dysplasia of the gastric mucosa as mild, moderate or severe (93). Riddell et al. developed a detailed grading system for inflammatory bowel diseases, which can be well used in classifying dysplastic changes in Barrett's oesophagus, since the morphologic spectrum is similar to that found in inflammatory bowel diseases (90,94). According to this system, mucosa is classified as negative, indefinite or positive for dysplasia. In mucosa classified as indefinite for dysplasia it is not possible to decide whether lesions are inflammatory, regenerative or neoplastic. Mucosal changes classified as positive for dysplasia are considered as neoplastic lesions, and are subdivided into low grade and high grade dysplasia. At initial endoscopic examination of patients with Barrett's oesophagus entering a surveillance programme, dysplasia is found in 11-37% of all cases without the presence of visible carcinoma (43,53,57,83,95,96). It has been demonstrated by prospective follow-up studies that additional 12-18% of the patients develop dysplasia during a mean follow-up period of 3 to 5.2 years (83,95,96). Ten to 25% of the patients with Barrett's oesophagus followed by serial endoscopy appear to progress from low grade dysplasia to high-grade dysplasia, and in the series of Hameeteman et al. even 67% of the patients with low-grade dysplasia developed high-grade dysplasia or adenocarcinoma. However, when low-grade dysplasia develops, progression to high-grade dysplasia and adenocarcinoma is not inevitable, and there are some reports of regression of dysplasia (95,96). Although high-grade dysplasia can persist for several years without progressing, the majority of patients with high-grade dysplasia will eventually develop adenocarcinoma (83,95,96). Dysplasia is found adjacent to adenocarcinoma in 68-100% and high-grade dysplasia in 35-84% of the patients undergoing oesophagectomy for Barrett's carcinoma (7,58,61,89,90,97-99). Moreover, high-grade dysplasia is an important marker for associated malignancy, since invasive adenocarcinoma is found in the resection specimens of 45-75% of the patients undergoing oesophagectomy for high-grade dysplasia (61,100-103).

The management of patients with high grade dysplasia without evidence of invasive cancer is controversial. Oesophagectomy with excision of all columnar lined epithelium is proposed by some investigators, whereas others favour continued follow-up using a rigorous, systematic endoscopic biopsy protocol until invasive cancer is detected (100-105). Undoubtedly, dysplasia in columnar lined oesophagus is the most important indicator of impending malignant change and at present the only reliable histopathological marker (56,61,85,105). The interobserver agreement for high grade dysplasia is 87%, and an interobserver agreement of 70% can be reached for indefinite or low grade dysplasia (106).

1.3.3 Environmental risk factors

Although smoking and alcohol consumption do not predispose to the development of metaplastic columnar lined oesophagus, smoking and to a lesser extent alcohol consumption are associated with the development of adenocarcinoma in patients with established Barrett's oesophagus (7,48,50,107).

1.3.4 Biomarkers for neoplastic progression in Barrett's oesophagus

Aneuploidy

Flowcytometric analysis of the DNA content of the epithelial nuclei provides objective information about the ploidy status and cell cycle events in populations of cells. In most series at least 90% of solid tissue cancers have undergone sufficient genomic changes to produce an aneuploid peak on flow cytometry (108). The morphologic stage in carcinogenesis at which aneuploidy develops is not clear, but in several precancerous lesions of various origin aneuploid cells have been demonstrated. In Barrett's oesophagus aneuploidy or increased G2/tetraploid fraction is associated not only with adenocarcinoma, but also with dysplasia in Barrett's epithelium (98,109-112). Although it has been observed in most series that the prevalence of aneuploid cell populations is increased as metaplasia progresses to dysplasia and carcinoma, discordance between flowcytometric abnormalities and dysplastic changes has been demonstrated (96,98,109,112). Aneuploidy is more commonly encountered than definite dysplasia, being found in non-dysplastic epithelium as well as in specimens with low and high

grade dysplasia (96). The presence of multiple different aneuploid populations of cells indicates more advanced genomic instability and has been shown to be strongly associated with dysplasia and adenocarcinoma in Barrett's oesophagus (96,98,113). Data from prospective follow-up studies indicated that aneuploidy and dysplasia were both prognosticators for the subsequent development of adenocarcinoma in patients with Barrett's oesophagus (96,113). Reid et al. demonstrated that 70% of the patients with aneuploidy or increased G2/tetraploid fractions in biopsy specimens obtained during initial endoscopic evaluation developed high-grade dysplasia or adenocarcinoma, whereas none of the patients without these abnormalities progressed to high-grade dysplasia or cancer (96). However, none of the patients with flowcytometric abnormalities on initial evaluation progressed to invasive cancer without first exhibiting high-grade dysplasia (96,113). Although flowcytometric abnormalities may be earlier and more specific markers for cancer development than dysplasia, flowcytometry seems not to provide sufficient additional information to justify its routine application in clinical practice so far (13).

Abnormal cell cyclus and proliferation

Several studies have demonstrated increased proliferative activity in Barrett's oesophagus by autoradiography, immunohistochemistry and DNA content flowcytometry (96,109,111). Autoradiographic studies reported expansion of the proliferative zone in columnar epithelium of patients with Barrett's oesophagus if dysplasia or carcinoma was present (114,115).

Immunohistochemical studies of Barrett's oesophagus showed increased proliferative fractions detected by monoclonal antibodies that recognize the proliferation associated antigens Ki-67 and proliferating cell nuclear antigen (PCNA) (116-118). The Ki-67 labelling index was found to be very low in junctional- or gastric type Barrett's epithelium, moderately high in intestinal type epithelium and very high in severe dysplastic epithelium or adenocarcinoma (119). In the study of Jankowski et al. the PCNA labelling index was higher in adenocarcinoma (25%) and in Barrett's intestinal type mucosa with high-grade dysplasia (26%) compared with intestinal type mucosa without significant dysplasia (20%) and Barrett's gastric type mucosa (12%) (118). Studies using DNA-content flow cytometry have demonstrated that the prevalence of increased S-phase and G2 fractions increase with advancing stages of neoplastic progression (96,109). Patients whose specimens taken at initial

examination had elevated S-phase and G2-fractions were at increased risk for progression to high-grade dysplasia and adenocarcinoma. A multiparameter flow cytometric assay showed that increased S-phase fractions developed in a subset of patients with Barrett's oesophagus and appeared to be associated with genomic instability, as evidenced by the high percentage of aneuploid cell populations that had increased S-phase fractions compared with diploid specimens (111). These studies indicate that neoplastic progression in Barrett's oesophagus is associated with the development of multiple cell cycle abnormalities, suggesting the sequential involvement of different cell cycle check points in the progression to malignancy in this condition.

Abnormal mucus and mucus production

It has been observed that there is reduced or absent mucus production within Barrett's dysplastic epithelium (106,112). Ultrastructural studies of Barrett's dysplastic epithelium demonstrated a distended rough endoplasmic reticulum, increased glycogen aggregates, and depletion of other cytoplasmic organelles required for mucus biosynthesis (112). Mucus granules were greatly diminished or absent. These abnormalities correlated with flowcytometric abnormalities and may result from defects in mucus biosynthesis that are related to neoplastic progression in Barrett's oesophagus. Lapertosa et al. demonstrated that O-acetylated sialomucins that were present in the goblet cells of specialized columnar epithelium were decreased markedly in the immediate vicinity of dysplasia, and absent in the dysplastic epithelium itself (120). They suggested that these results indicate a relative tissue immaturity that might be useful as an early sign of malignancy. However, as yet there are no data on the sensitivity or specificity of these changes for detection of dysplasia or identification of patients at increased risk for oesophageal adenocarcinoma.

Mucosal enzymes

Ornithine decarboxylase (ODC) is a key enzyme in the biosynthetic pathway for polyamines and is important in cell proliferation and differentiation. In Barrett's oesophagus ODC activity is increased in some cases of dysplasia, but a direct relation between ODC activity and polyamine content could not be demonstrated (121-123). Moreover, the distinction between specialized and dysplastic columnar lined oesophagus could not be made by measuring the polyamine content (124).

Carcinoembryonic antigen (CEA)

CEA has been identified as a glycoprotein present in fetal gut, normal colonic mucosa and in many neoplasms, especially those of the gastrointestinal tract. CEA expression has been found in Barrett's oesophagus, mainly in intestinal type epithelium (125). However, a direct relation with malignant degeneration could not be demonstrated.

Growth regulatory factors

Gastrointestinal mucosa may synthesize epidermal growth factor (EGF) and transforming growth factor (TGF-alpha) which bind to epidermal growth factor receptors (EGF-R) and may act as autocrine, paracrine and humoral growth factors in regulating the growth and differentiation of the gastrointestinal tract (126). It has been noted that the expression of growth factors and their receptors correlates with the degree of mucosal dysplasia of the oesophagus, as well as with the occurrence of oesophageal adenocarcinoma (118,119,127,128). Co-expression of EGF or TGF-alpha and EGF-R is associated with autocrine growth regulation in oesophageal carcinoma cells (128). Barrett's mucosa, especially when dysplastic, and Barrett's adenocarcinoma overexpress TGF-alpha and EGF-R, indicating the possibility of autocrine growth regulation (119). TGF-alpha and EGF-R expression were found to be low in junctional- or gastric type Barrett's epithelium, and high in intestinal type epithelium, Barrett's epithelium with moderate to severe dysplasia and adenocarcinoma (118,119,127). EGF expression is unaffected by the degree of dysplasia in Barrett's epithelium. A significant correlation between the intensity of TGF-alpha and EGF-R expression and Ki-67 and PCNA labelling indices has been shown. Although the expression of growth factors and their receptors is significantly greater in intestinal type Barrett's metaplasia and dysplastic epithelium compared with normal mucosa, its independent prognostic value has not been proven by prospective studies.

Chromosomal abnormalities

There is substantial evidence that the progression to malignancy in Barrett's oesophagus is associated with a multistep process of genetic instability and clonal evolution (96,98,129). There are a few reports of cytogenetic analysis of Barrett's epithelium and adenocarcinoma (130-132). Karyotypes are often complex and contain multiple numerical and structural rearrangements. Barrett's metaplasia can contain cytogenetically abnormal clones

that occupy extensive regions of the Barrett's segment, persist for several years, and progress to high-grade dysplasia and adenocarcinoma (131). The most consistent numerical chromosomal aberration found in cytogenetic studies of dysplastic columnar mucosa and adenocarcinoma is loss of the Y-chromosome (130-132). In a cytogenetic study of 9 gastric and lower oesophageal adenocarcinomas, of which 3 had developed in Barrett's oesophagus, non-random structural rearrangements involving the region 11p13-15 were detected in 8 (132).

It has been shown that the development of Barrett's adenocarcinoma is associated with allelic losses that include tumour suppressor genes (133). Allelic losses of 17p occur in diploid cells as early events and typically precede the development of aneuploidy and other allelic losses during neoplastic progression in Barrett's oesophagus (134). There is strong evidence that the target of 17p allelic losses is the p53 gene, a tumour suppressor gene found to be lost or mutated in many tumours (129,135,136). Allelic losses of 17p or 5q were found in aneuploid cell populations from patients with Barrett's oesophagus who had high-grade dysplasia or cancer (129). Allelic losses of 17p were found in 92% of Barrett's adenocarcinoma and 5q allelic losses in 77% (135,137). It was demonstrated that 17p allelic losses typically occurred before 5q allelic losses during neoplastic progression in Barrett's oesophagus (129,134). Allelic losses of 5q in Barrett's adenocarcinoma involve both APC (adenomatosis polyposis coli) and MCC (mutated in colon cancer) genes in the majority of tumours. In addition, allelic losses of 13q, 18q and 8p have been found (133).

Microsatellite instability is another form of genomic instability found in both Barrett's metaplasia and in approximately one-fourth of Barrett's associated adenocarcinomas (138). Microsatellites are short repeated nucleotide sequences interspersed throughout the human genome (139). In a subset of tumours, errors in DNA repair occur, resulting in completely different lengths of DNA in these regions. Microsatellite instability can develop as an early event in metaplasia and in diploid tumour cells, before aneuploidy occurs (138). Secondary microsatellite alterations have been found in aneuploid DNA, suggesting that these abnormalities continue to accumulate as the neoplastic process progresses.

Tumour suppressor genes and (proto)oncogenes

P53 is a component of a signal transduction pathway that responds to DNA damage by causing cells to arrest in G1 (140). Cells that lack p53 enter the S-phase inappropriately

and are predisposed to genomic instability (141). Mutations in the p53 gene frequently prolong the half life of p53 protein, rendering it detectable by immunohistochemistry and multiparameter flowcytometry. The presence of p53 protein immunostaining in Barrett's adenocarcinoma ranges from 53-87% (135,142-146). Ramel et al. demonstrated progressive increase in the percentage of patients with p53 overexpression with increasing histological risk of malignancy: 5% of the patients with non-dysplastic Barrett's epithelium showed p53 overexpression, 15% of those with indefinite or low-grade dysplasia, 45% of those with high-grade dysplasia and 53% of those with Barrett's adenocarcinoma (143). The prevalence of p53 protein overexpression is higher in aneuploid cell populations as compared to diploid cell populations of high-grade dysplasia and adenocarcinoma in Barrett's oesophagus. Similar results were published by Younes et al. (144). Flejou et al. found that p53 overexpression was limited to carcinoma and high-grade dysplasia, suggesting that p53 is a relatively late event in the neoplastic transformation of Barrett's oesophagus (145). Since not all patients with p53 overexpression in Barrett's epithelium progress to carcinoma and on the other side a subset of patients with Barrett's oesophagus will undergo malignant transformation despite lack of immunostaining for p53 protein, the value of p53 as a marker for identifying patients with Barrett's oesophagus at high risk of developing adenocarcinoma is unclear so far.

Allelic losses of other chromosomal regions harbouring known tumour suppressor genes have been found in oesophageal adenocarcinomas: allelic losses of Rb (13q) in 42%, DCC (18q) in 38%, MCC (5q) in 83% and APC (5q) in 80% (133).

Expression of the c-erbB2 proto-oncogene, encoding a transmembrane tyrosine kinase receptor highly homologous to EGF-R, has been demonstrated in Barrett's mucosa and adenocarcinoma (147-149). Specimens of Barrett's oesophagus with severe dysplastic changes showed increased expression of c-erbB2 protein on the cell membranes. The mechanism of overexpression of c-erbB2 protein in most cases results from gene amplification.

Ras oncogenes play a role in cell growth and differentiation (147). H-ras mutations are consistently associated with dysplastic Barrett's mucosa and adenocarcinoma, but are not found in non-dysplastic Barrett's metaplasia (150).

In the study of Meltzer et al. abundant c-myc expression was found in Barrett's epithelium and oesophageal adenocarcinoma, but Jankowski et al. could not demonstrate c-myc in either Barrett's mucosa or adenocarcinoma (142,151).

To a lesser extent expression of the oncogenes c-srs (encoding a cytoplasmic protein

with tyrosine kinase activity) and c-jun (controlling the number of cell divisions) was found in Barrett's epithelium and adenocarcinoma (142).

It is likely that the synchronous expression of oncogenes may act synergistically, gradually overcoming the negative feedback regulation of mitogenesis. However, the prognostic value of these oncogenes has to be assessed in prospective studies.

1.4 Adenocarcinoma in Barrett's oesophagus

1.4.1 Prevalence and incidence

There has been a large increase in the incidence of oesophageal adenocarcinoma in the last two decades in the United States and Europe (152-156). From 1926 to 1976 adenocarcinoma represented only 0.8 to 3.7% of oesophageal cancers, while reports in the 1980's showed an increase in the proportion of oesophageal cancer represented by adenocarcinoma to 18-50% (152,157,158). The annual age- and sex-adjusted incidence of oesophageal adenocarcinoma rose from 0.13 in 1935-1971 to 0.74 cases per 100,000 in 1974-1989 in the United States, and from 0.18 in 1967-1971 to 0.63 cases per 100,000 in 1977-1981 in the United Kingdom (152,159). There is a higher incidence rate of oesophageal adenocarcinoma among white males as compared to black males and white females, but comparable increases in the incidence rates have been observed (153). Adenocarcinomas of the oesophagus are found to be associated with Barrett's oesophagus in 41-86% of the cases (88,152,154,156,158,160). However, this may be underestimated since Barrett's oesophagus is most often defined as columnar epithelium extending more than 2-3 cm above the gastro-oesophageal junction, and in some cases adenocarcinoma may arise in small areas or tongues of specialized epithelium (161). Retrospective and prospective follow-up studies reported an incidence of adenocarcinoma in Barrett's oesophagus varying from one in 52 to one in 441 patient-years of follow-up, with a mean follow-up period ranging from 2.9 to 15 years (43,48,50,83-85,92,162). Possible explanations for the variation in incidence are the fact that some studies are retrospective, there may be a referral bias, and methods of follow-up may differ (43,163). Compared with the incidence of oesophageal carcinoma in the general population, the incidence of adenocarcinoma arising in patients with Barrett's oesophagus is 30 to 125 times greater (43,48,50,83-85,162).

1.4.2 Clinical presentation and diagnosis

The average age at the time of diagnosis of an adenocarcinoma in Barrett's oesophagus is 57-63 years, although the age ranges from 23 to 96 years (7,34,41,44,77,89,97,156). There is a strong male predominance with a male to female ratio ranging from 3:1 to 15:1. The predominant presenting symptom is dysphagia, occurring in 60-87% of patients with an adenocarcinoma in Barrett's oesophagus (7,34,41,44,48,89,97,164). Other symptoms that occur in a minority of patients are weight loss (42%), hemorrhage (12-36%) and pain (25%). Of all patients with an adenocarcinoma in Barrett's oesophagus, only 5-29% was known to have a Barrett's oesophagus before the development of carcinoma (97,156). This suggests that an unknown proportion of the general population probably has Barrett's oesophagus, but remains undetected unless carcinoma develops and symptoms related to malignancy lead to the discovery of both adenocarcinoma and Barrett's oesophagus simultaneously (33,48,50).

An advanced adenocarcinoma in Barrett's oesophagus may appear on double-contrast oesophagography as a stricture, protruding tumour mass or ulcer (164,165). The endoscopic diagnosis of carcinoma of the oesophagus is usually straightforward when the tumour is in an advanced stage, as is often the case when a patient presents with complaints of dysphagia. Adenocarcinomas in Barrett's oesophagus are nodular or polypoid and less frequently ulcerating (166). Diffuse infiltration, presenting as a thickened and rigid oesophageal wall may be present. In most cases there is narrowing of the oesophageal lumen and submucosal tumour extension. There is a better chance of detecting early carcinomas by endoscopy as compared to radiologic techniques. However, there may be no grossly recognizable neoplastic lesions at all at endoscopy (56,83). Therefore, multiple biopsies should be obtained not only from any grossly visible lesions, but also at small intervals throughout the length of the columnar lined oesophagus. Some authors proposed to use cytologic brushings, as the use of oesophageal brush cytology and biopsy specimens provides two complementary techniques, which detect a greater number of malignant lesions than either technique alone (167,168).

The diagnosis adenocarcinoma in Barrett's oesophagus has to be confirmed by histologic examination. If the bulk of the tumour is situated in the oesophagus and has a patch of Barrett's mucosa at its margin, the diagnosis of Barrett's carcinoma is assured. In 80% the tumours are located in the lower third of the oesophagus (88). Tumours grow infiltrative or expansive, they are well to poorly differentiated and often present multifocally (58,102,169). Although the carcinomas that arise in Barrett's mucosa are adenocarcinomas in the vast

majority, other types of cancers occurring in Barrett's oesophagus have been described. Adenosquamous carcinomas and squamous cell carcinomas of the oesophagus, most often located in the native squamous lined oesophageal mucosa proximal to the columnar lined segment, were found to be associated with Barrett's oesophagus (170-172).

1.4.3 Treatment and survival

The treatment of choice most often reported for adenocarcinoma of the oesophagus is surgical resection (7,34,41,44,89,97,164). If the general condition of the patient is good enough to undergo surgical treatment, further analysis has to be performed to assess whether a lesion is potentially resectable and to exclude the presence of distant metastases. The tumour extension and depth of oesophageal wall invasion can be analyzed by various examinations, including endoscopy, radiology, computed tomography, nuclear magnetic resonance imaging (MRI), and endoscopic ultrasonography (173-178). The invasion of adjacent structures and depth of tumour invasion can correctly be assessed by computer tomography scanning in more advanced stages, but it is inaccurate in staging early carcinoma of the oesophagus (174-176). The experience with MRI is similar (179). Endoscopic ultrasonography has been shown to be useful in the preoperative staging of oesophageal carcinoma, and even in early carcinomas there is an accuracy of 81.5-90% in T-staging (177,178). Diagnostic evaluation of a possible infiltration of the tracheobronchial system can best be performed by bronchoscopy (173). Pretreatment lymph node staging can be performed by computer tomography, MRI or endoscopic ultrasonography (173,177,180). Endoscopic ultrasonography leads to the best results in assessing mediastinal lymph node metastasis (accuracy 78.4-82%) as compared to computed tomography (accuracy 56-77%). Distant lymph node metastases, supraclavicular and abdominal, can be assessed by computed tomography, ultrasound or both, and the diagnosis can be confirmed histologically by ultrasound-guided fine-needle aspiration biopsy (181,182). Preoperative staging can finally be performed by diagnostic laparoscopy combined with laparoscopic ultrasonography (183).

The aim of surgical treatment of an oesophageal carcinoma is to achieve locoregional freedom of tumour (156,184). The standard oesophagectomy consists of a subtotal oesophagectomy and sampling of obvious lymph nodes, through a right or left thoracoabdominal approach or using the technique of transhiatal oesophagectomy avoiding

thoracotomy (156,158,185). Some authors reported better survival rates after en bloc resection of the oesophageal tumour completely covered on all sides by normal tissue and incorporating all the potentially involved peritumoural and regional lymph nodes as compared to survival rates after standard oesophagectomy (158,186). Reconstruction can be achieved using a stomach tube or a colon interposition (156,158,184,185). The postoperative mortality within 30 days after oesophagectomy for carcinoma lies between 0 and 6.5% in specialized centers, even after the more extensive en bloc resection (97,158,184-186). Postoperative morbidity up to 50% has been reported, but varies in most series between 20% and 30%. Pulmonary complications are the most frequent (23-35%), followed by recurrent laryngeal nerve paresis (7-31%) after a cervical anastomosis, anastomotic leakage (2-17%), postoperative bleeding (3-7%) and wound infection (5-8%). There is no effect of adjuvant chemotherapy on the survival of patients with an adenocarcinoma of the oesophagus and cardia, and prospective randomized trials showed no effect of preoperative radiotherapy either (184,187,188). Patients with metastatic disease or patients who are not fit enough to undergo oesophagectomy can be treated for palliation. Symptoms based on obstructing oesophageal cancer can be treated by intraluminal irradiation, laser therapy or electrocoagulation of the tumour (189-191). Palliation of a malignant stricture by intubation is performed in patients with end stage disease (156,192).

The survival for patients with an adenocarcinoma in Barrett's oesophagus is poor, even after surgical treatment. Overall 5-year survival after oesophagectomy ranges from 15% to 58% (7,97,164,186). Survival is influenced by the depth of tumour penetration into the oesophageal wall and the presence of lymph node metastasis. The reported 5-year survival rates after surgical treatment for superficial oesophageal carcinoma, i.e. tumours restricted to the mucosa and submucosa (Tis and T1 tumours according to the TNM-classification) range from 63% to 100% (97,184,186,193,194). Metastasis to lymph nodes and vessel invasion develop rarely in intramucosal tumours, but occur frequently in submucosal tumours (35-40%), resulting in a significant difference in the 5-year survival of patients with an intramucosal tumour (84-100%) versus patients with a submucosal tumour (55-64%) (193,195). The lymph node status is an important prognostic index since the 5-year survival rates of patients without lymph node metastasis varies between 30-85% compared to 0-38% for patients with lymph node metastasis (97,196). More favourable survival rates have been shown after more radical resections and extensive lymphadenectomies, although no

prospective randomized studies are reported.

1.5 Aims of the studies

Barrett's oesophagus is a premalignant condition. In comparison with the general population patients with Barrett's oesophagus run a 30 to 125 fold increased risk of developing an oesophageal adenocarcinoma. Once a carcinoma has developed, prognosis is poor. Since locoregional freedom of tumour by surgical treatment of oesophageal carcinoma is more likely to be achieved in earlier stages of cancer, there is an indication that early diagnosis will benefit the patient. It is therefore important to determine if tumour stage, including the depth of infiltration and the presence of lymph node metastases, and the differentiation grade of the tumour affect the survival of patients with an adenocarcinoma in Barrett's oesophagus. We performed a retrospective study to analyze the survival of patients with an adenocarcinoma in columnar lined oesophagus in relation to surgical treatment, stage and differentiation grade of the tumour (Chapter 2.1).

Early diagnosis of oesophageal adenocarcinoma can be achieved by endoscopic surveillance of patients with Barrett's oesophagus. Instead of submitting all patients with Barrett's oesophagus, which is time consuming and may not be cost-effective, it would be more efficient to select a subgroup of patients at high risk of developing carcinoma for endoscopic screening. To identify this subset of patients, it is necessary to define factors which indicate an increased risk of neoplastic progression. The significance of clinical characteristics like sex, age, smoking and drinking habits and the extension of Barrett's oesophagus as risk factors for neoplastic progression were evaluated in Chapter 3.

The only histopathological marker for impending malignant change in clinical use at the moment is the presence and grade of dysplasia. Malignancies develop in association with genomic instability, resulting in quantitative and qualitative aberrations. Quantitative abnormalities of the DNA content can be assessed by flowcytometry. The association of dysplasia and aneuploidy with malignant change in Barrett's oesophagus was analyzed by a case control study (Chapter 4). Furthermore, the influence of DNA-ploidy of tumour cells on patient survival was studied (Chapter 2.2).

Finally, we studied genomic abnormalities by cytogenetic analysis (Chapter 5). If there are chromosomal aberrations common to all oesophageal adenocarcinomas, occurring in early stages of neoplastic progression, genetic analysis would be helpful in selecting patients with Barrett's oesophagus at high risk. Therefore, we studied genomic abnormalities in

adenocarcinoma of the distal oesophagus and cardia by cytogenetic analysis to discover specific chromosomal rearrangements. Barrett's mucosa was also analyzed cytogenetically to investigate if chromosomal abnormalities occurred as an early event before invasive tumour could be demonstrated.

1.6 References

1. Barrett NR. Chronic peptic ulcer of the oesophagus and oesophagitis. Br J Surg 1950;38:175-182.
2. Tilestone W. Peptic ulcer of the esophagus. Am J Med Sci 1906;132:240-265.
3. Lyall A. Chronic peptic ulcer of the oesophagus. A report of eight cases. Br J Surg 1937;24:534-547.
4. Allison PR, Johnstone AS. The oesophagus lined with gastric mucous membrane. Thorax 1953;8:87-101.
5. Barrett NR. The lower esophagus lined by columnar epithelium. Surgery 1957;41:881-894.
6. Lortat-Jacob JL. L'endo-brachy-oesophage. Ann Chir 1957;11:1247-1254.
7. Skinner DB, Walther BC, Riddell RH, Schmidt H, Iascone C, DeMeester TR. Barrett's esophagus: Comparison of benign and malignant cases. Ann Surg 1983;198:554-566.
8. Johns BAE. Developmental changes in the esophageal epithelium in man. J Anat 1952;86:431-442.
9. Prior A, Whorwell PJ. Familial Barrett's esophagus ? Hepato-Gastroenterol 1986;33:86-87.
10. Crabb DW, Berk MA, Hall TR, et al. Familiar gastroesophageal reflux and development of Barrett's esophagus. Ann Intern Med 1985;103:52-54.
11. Jochem VJ, Fuerst PA, Frankes JJ. Familial Barrett's esophagus associated with adenocarcinoma. Gastroenterology 1992;102:1400-1402.
12. Everhart CW, Holtzapple PG, Humphries TJ. Barrett's esophagus: inherited epithelium or inherited reflux ? J Clin Gastroenterol 1983;5:357-360.
13. Cameron AJ. Barrett's esophagus and adenocarcinoma: from the family to the gene. Gastroenterology 1992;102:1421-1424.
14. Goldman MC, Beckman RC. Barrett syndrome, case report with discussion about concepts of pathogenesis. Gastroenterology 1960;39:104-110.
15. Adler RH. The lower esophagus lined by columnar epithelium; its association with hiatal hernia, ulcer, stricture and tumor. J Thorac Cardiovasc Surg 1963;45:13-32.

16. Agha FP, Keren DF. Barrett's esophagus complicating achalasia after esophagomyotomy. J Clin Gastroenterol 1987;9:232-237.

17. Hamilton SR, Yardley JH. Regeneration of cardiac type mucosa and acquisition of Barrett mucosa after esophagogastrostomy. Gastroenterology 1977;72:669-675.

18. Katzka DA, Reynolds JC, Saul SH et al. Barrett's metaplasia and adenocarcinoma of the esophagus in scleroderma. Am J Med 1987;82:46-52.

19. Karl TR, Pindick F, Sicular A. Zollinger-Ellison syndrome with esophagitis and Barrett's mucosa. Am J Gastroenterol 1983;78:611-614.

20. Bremner CG, Lynch VP, Ellis FH. Barrett's esophagus: congenital or acquired ? Surgery 1970;68:209-216.

21. Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TPJ. Experimental columnar metaplasia in the canine oesophagus. Br J Surg 1988;75:113-115.

22. Iascone C, DeMeester TR, Little AG, Skinner DB. Barrett's esophagus: Functional assessment, proposed pathogenesis and surgical therapy. Arch Surg 1983;118:543-549.

23. Gillen P, Keeling P, Byrne PJ, Hennessy TPJ. Barrett's oesophagus: pH profile. Br J Surg 1987;74:774-776.

24. Hooloway RH, Dobss WJ. Esophageal motor function in Barrett's esophagus. In: Barrett's esophagus: pathophysiology, diagnosis and management. Spechler SJ, Goyal RK (eds). New York, Elsevier Science 1985,vol 1, pp105-112.

25. Mulholland M, Reid BJ, Levine DS, et al. Elevated gastric acid secretion in patients with Barrett's metaplastic epithelium. Dig Dis Sci 1989;9:1329-1335.

26. Johnson DA, Winters C, Spurling TJ, Chobanian SJ, Cattau EL Jr. Esophageal acid sensitivity in Barrett's esophagus. J Clin Gastroenterol 1987;9:23-27.

27. Dent J, Bremner CG, Collen MJ, et al. Barrett's oesophagus. Working party report. J Gastroenterol Hepatol 1991;6:1-22.

28. Gillen P, Keeling P, Byrne PJ, Healy M, O'Moore RR, Hennessy TPJ. Implication of duodenogastric reflux in the pathogenesis of Barrett's oesophagus. Br J Surg 1988;75:540-543.

29. Attwood SEA, Ball CS, Barlow AP, Jenkinson L, Norris TL, Watson A. Role of intragastric and intraoesophageal alkalinisation in the genesis of complications in Barrett's columnar lined lower oesophagus. Gut 1993;34:11-15.

30. Seto Y, Kobori O. Role of reflux oesophagitis and acid in the development of columnar epithelium in the rat oesophagus. Br J Surg 1993;80:467-470.

31. Spechler SJ, Schimmel EM, Dalton JW, Doos W, Tries JS. Barrett's epithelium complicating lye ingestion with sparing of the distal esophagus. Gastroenterology 1981;81:580-583.

32. Dahms BB, Greco MA, Stradjord SE, Rothsteir FC. Barrett's esophagus in three children after antileukemia chemotherapy. Cancer 1987;60:2896-2900.

33. Cameron AJ, Zinsmeister AR, Ballard DJ, Carrey JA. Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings. Gastroenterology 1990;99:918-922.

34. Starnes VA, Adkins RB, Ballinger JF, Sawyers JL. Barrett's esophagus. A surgical entity. Arch Surg 1984;119:563-567.

35. Winters C, Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, et al. Barett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-124.

36. Mann NS, Tsai MF, Nair PK. Barrett's esophagus in patients with synptomatic reflux esophagitis. Am J Gastroenterol 1989;84:1494-1496.

37. Spechler SJ, Sperber H, Doos WG, et al. The prevalence of Barrett's esophagus in patients with chronic peptic esophageal strictures. Dig Dis Sci 1983;28:769-774.

38. Borrie J, Goldwater L. Columnar cell-lined esophagus: assessment of etiology and treatment. A 22 year experience. J Thorac Cardiovasc Surg 1976;71:825-834.

39. Polepalle SC, McCallum RW. Barrett's esophagus: current assessment and future perspectives. Gastroenterol Clin North Am 1990;19:733-744.

40. Cameron AJ, Lomboy CT. Barrett's esophagus: age, prevalence and extent of columnar epithelium. Gastroenterology 1992;103:1241-1245.

41. Sarr MG, Hamilton SR, Marrone GC, Cameron JL. Barrett's esophagus: it's prevalence and association with adenocarcinoma in patients with symptoms of gastroesophageal reflux. Am J Surg 1985;149:187-193.

42. Cooper BT, Barbezat GO. Barrett's oesophagus. A clinical study of 52 patients. Q J Med 1987;62:97-108.

43. Williamson WA, Ellis FH Jr, Gibb SP,et al. Barrett's esophagus: prevalence and incidence of adenocarcinoma. Arch Intern Med 1991;151:2212-2216.

44. Sjogren RW, Johnson LF. Barrett's esophagus: A review. Am J Med 1983;74:313-321.

45. DeMeester TR, Attwood SE, Smyrk TC, Therkildsen DH, Hinder RA. Surgical therapy in Barrett's esophagus. Ann Surg 1990;212:528-541.

46. Mangla JC. Barrett's esophagus: an old entitiy rediscovered. J Clin Gastroenterol 1981;3:347-356.

47. Carrie A. Adenocarcinoma of the upper end of the oesophagus arising from ectopic gastric epithelium. Br J Surg 1950;37:474...

48. Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. N Engl J Med 1985;313:857-859.

49. Naef AP, Savary M. Columnar-lined oesophagus: an acquired lesion with malignant predisposition. J Thorac Cardiovasc Surg 1975;70:826-835.

50. Spechler SJ, Robbins AH, Bloomfield Rubins H, Vincent ME, Heeren T, et al. Adenocarcinoma and Barrett's oesophagus. An overrated risk ? Gastroenterology 1984;87:927-933.

51. Davidson JS. High peptic strictures of the oesophagus. Thorax 1976;31:1-14.

52. Sontag SJ, Schnell TG, Chejfec G et al. Barrett's oesophagus and colonic tumours. Lancet 1985;27:946-949.

53. Herlihy KJ, Orlando RC, Bryson JC, et al. Barrett's esophagus: clinical, endoscopic, histologic, manometric and electrical potential difference characteristics. Gastroenterology 1984;86:436-443.

54. Woolf GM, Riddell RH, Irvine EJ, et al. A study to examine agreement between endoscopy and histology for the diagnosis of columnar lined (Barrett's) esophagus. Gastrointest Endosc 1989;35:541-544.

55. Belladona JA, Hajdu SI, Bains MS, et al. Adenocarcinoma in situ of Barrett's esophagus diagnosed by endoscopic cytology. N Engl J Med 1974;291:895-896.

56. Reid BJ, Weinstein WM, Lewin KJ, Haggitt R, VanDeventer G, DenBesten L, et al. Endoscopic biopsy can detect high-grade dysplasia or early adenocarcinoma in Barrett's esophagus with or without grossly recognizable neoplastic lesions. Gastroenterology 1988;94:81-90.

57. Paull A, Trier JS, Dalton MD, Camp RC, Loeb P, Goyal RK. The histologic spectrum of Barrett's esophagus. N Engl J Med 1976;295:476-480.

58. Thompson JJ, Zinsser KR, Enterline HT. Barrett's metaplasia and adenocarcinoma of the esophagus and gastroesophageal junction. HUM Pathol 1983;14:43-61.

59. Levine DS, Rubin CE, Reid BJ, Haggitt RC. Specialized metaplastic columnar epithelium in Barrett's esophagus. Lab Invest 1989;3:418-432.

60. Das KM, Prasad I, Garla S, Amenta PS. Detection of a shared colon epithelial epitope on Barrett epithelium by a novel monoclonal antibody. Ann Int Med 1994;120:753-756.

61. Hamilton SR, Smith RR. The relationship between columnar epithelial dysplasia and invasive adenocarcinoma arising in Barrett's esophagus. Am J Clin Pathol 1987;87:301-312.

62. Hameeteman W. Columnar-lined (Barrett's) esophagus (Thesis,1989). Chapter 3: Study of histology and dysplasia in columnar-lined esophagus.

63. Takubo K, Sasajima K, Yamashita K, Tanaka Y, Fujita K. Double muscularis mucosae in Barrett's esophagus. Hum Pathol 1991;22:1158-1161.

64. Garrett WR, Dukes GE. Upper gastrointestinal disorders. In: Applied Therapeutics. Young L, Koda-Kimble MA (eds), 5th ed. Seattle, 1992.

65. Streitz JM, Williamson WA, Ellis FH Jr. Current concepts concerning the nature and treatment of Barrett's esophagus and its complications. Ann Thorac Surg 1992;54:586-591.

66. Decktor DL, Robinson MG. Gastroesophageal reflux: clinical diagnosis, current therapy, future trends. Hepato-Gastroenterol 1990;379suppl1):11-17.

67. Collen MJ, Lewis JH, Benjamin SB. Gastric acid hypersecretion in refractory gastroesophageal reflux disease. Gastroenterology 1990;98:645-661.

68. Wesdorp ICE, Bartelsman J, Schipper MEI, Tytgat GN. Effect of long-term treatment with cimetidine and antacids in Barrett's oesophagus. Gut 1981;22:724-727.

69. Cooper BT, Barbezat GO. Treatment of Barrett's esophagus with H2 blockers. J Clin Gastroenterol 1987;9:139-141.

70. Lee FI, Isaacs PET. Barrett's ulcer: response to standard dose ranitidine, high dose ranitidine and omeprazole. Am J Gastroenterol 1988;83:914-916.

71. Klinkenberg-Knol EC, Jansen JMBJ, Festen HPM, Meuwessen SGM, Lamers CBHW. Double blind multicentre comparison of omeprazole and ranitidine in the treatment of reflux oesophagitis. Lancet 1987;1:349-351.

72. Hameeteman W, Tytgat GN. Healing of chronic Barrett ulcers with omeprazole. Am J Gastroenterol 1986;81:764-766.

73. Atkinson M, Robertson CS. Benign oesophageal stricture in Barrett's columnar epithelialised oesophagus and its responsiveness to conservative management. Gut 1988;29:1721-1724.

74. Sampliner RE. Effect of up to 3 years of high-dose lansoprazole on Barrett's esophagus. Am J Gastroenterol 1994;89:1844-1848.

75. Sampliner RE, Garewal HS, Fennerty MB, Aickin M. Lack of impact of therapy on extent of Barrett's esophagus in 67 patients. Dig Dis Sci 1990;35:93-96.

76. Patel GK, Clifft SA, Schaeffer RA, Read RC, Texter EC. Resolution of severe dysplastic (Ca in situ) changes with regression of columnar epithelium in barrett's esophagus on medical treatment. Gastroenterology 1982;82:1147A.

77. Gore S, Healy CJ, Sutton R, Eyre-Brook IA, Gear MW, Shepherd NA, Wilkinson SP. Regression of columnar lined (Barrett's) oesophagus with continuous omeprazole therapy. Alimentary Pharmacology & Therapeutics 1993;7:623-628.

78. Berenson MM, Johnson TD, Markowitz NR, Buchi KN, Samowitz WS. Restoration of squamous mucosa after ablation of Barrett's esophageal epithelium. Gastroenetrology 1993;104:1686-1691.

79. Fink MA, Martin CJ, Ewing HP, Thompson ME, Machet D, Rode J. Reversal of experimental Barrett's esophagus by endoscopic laser ablation and reduction of acid reflux. Gastroenterology 1992;102:A924.

80. Williamson WA, Ellis FH Jr, Gibb SP, Shahian DM, Aretz HT. Effect of antireflux operation on Barrett's mucosa. Ann Thorac Surg 1990;49:537-542.

81. Ogorek CP, Fisher RS. Detection and treatment of gastroesophageal reflux disease. Gastroenterol Clin n Am 1989;18:293-313.

82. Attwood SEA, Barlow AP, Norris TL, Watson A. Barrett's oesophagus: effect of antireflux surgery on symptom control and development of complications. Br J Surg 1992;79:1050-1053.

83. Hameeteman W, Tytgat GNJ, Houthoff HJ, et al. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989;96:1249-1256.

84. Van der Veen AH, Dees J, Blankensteijn JD, van Blankenstein M. Adenocarcinoma in Barrett's oesophagus: an overrated risk. Gut 1989;30:14-18.

85. Robertson CS, Mayberry JF, Nicholson DA, James PD, Atkinson M. Value of endoscopic surveillance in the detection of neoplastic change in Barrett's oesophagus. Br j Surg 1988;75:760-763.

86. Streitz JM, Andrews CW, Ellis FH. Endoscopic surveillance of Barrett's oesophagus. Does it help? J Thorac Cardiovasc Surg 1993;105:383-388.

87. Spechler SJ. Endoscopic surveillance for patients with Barrett esophagus: does the cancer risk justify the practice? Ann Int Med 1987;106:902-903.

88. Hamilton SR, Smith RRL, Cameron JL. Prevalence and characteristics of Barrett esophagus in patients with adenocarcinoma of the esophagus or esophagogastric junction. Hum Pathol 1988;19:942-948.

89. Harle IA, Finley RJ, Belsheim M, Bondy DC, Booth M, Lloyd D, et al. Management of adenocarcinoma in a columnar-lined esophagus. Ann Thorac Surg 1985;40:330-336.

90. Schmidt HG, Riddell RH, Walther B, Skinner DB, Riemann JF. Dysplasia in Barrett's esophagus. J Cancer Res Clin Oncol 1985;110:145-152.

91. Ransom JM, Patel GK, Clift SA, Wamble NE, Read RC. Extended and limited types of Barrett's esophagus in the adult. Ann Thorac Surg 1982;33:19-27.

92. Iftikhar SY, James PD, Steele RJC, Hardcastle JD, Atkinson M. Length of Barrett's oesophagus: an important factor in the development of dysplasia and adenocarcinoma. Gut 1992;33:1155-1158.

93. Morson BC, Solim LH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol 1980;33:711-721.

94. Riddell RH, Goldmann H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, et al. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum Pathol 1983;14:931-968.

95. Miros M, Kerlin P, Walker N. Only patients with dysplasia progress to adenocarcinoma in Barrett's oesophagus. Gut 1991;32:1441-1446.

96. Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC, Rabinovitch PS. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenterology 1992;102:1212-1219.

97. Streitz JM, Ellis FH Jr., Gibb SP, Balogh K, Watkins E. Adenocarcinoma in Barrett's esophagus. A clinicopathological study of 65 cases. Ann Surg 1991;213:122-125.

98. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett's esopahgus is associated with genomic instability. Lab Invest 1988;60:65-71.

99. Smith RR, Hamilton SR, Boitnott JR, et al. The spectrum of carcinoma arising in Barrett's esophagus. A clinicopathologic study of 26 patients. Am J Surg Pathol 1984;8:563-573.

100. Altorki NK, Sunagawa M, Little AG, Skinner DB. High-grade dysplasia in the columnar-lined esophagus. Am J Surg 1991;161:97-100.

101. Pera M, Trastek VF, Carpenter HA, Allen MS, Deschamps C, Pairolero PC. Barrett's esophagus with high-grade dysplasia: an indication for esophagectomy? Ann Thorac Surg 1992;54:199-204.

102. McArdle JE, Lewin KJ, Randall G, Weinstein W. Distribution of dysplasia and early invasive carcinoma in Barrett's esophagus. Human Pathol 1992;23:479-482.

103. Putnam JB, Soysal O, Roth JA, Walsh GC, Nessbitt JC, Schrump DS. Results of esophagectomy for severe dysplasia in Barrett's esophagus (Abstract). Sixth World congress of the ISDE. Milan, Italy, August 23-26, 1995.

104. Rush VW, Levine DS, Haggitt R, Reid BJ. The management of high grade dysplasia and early cancer in Barrett's esophagus. A multidisciplinary problem. Cancer 1994;74:1225-1229.

105. Levine DS, Haggitt RC, Blount PC, Rabinovitch PS, Rush VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993;105:40-50.

106. Reid BJ, Haggitt RC, Rubin CE, Roth G, Surawicz CM. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. Hum Pathol 1988;19:166-178.

107. Gray MR, Donnelly RJ, Kingsnorth AN. The role of smoking and alcohol in metaplasia and cancer risk in in Barrett's columnar lined oesophagus. Gut 1993;34727-731.

108. Friedlander ML, Hedley DW, Taylor IW. Clinical and biological significance of aneuploidy in human tumors. J Clin Pathol 1984;37:961-974.

109. Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. Barrett's esophagus. Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. Gastroenterology 1987;93:1-11.

110. Fennerty MB, Sampliner RE, Way D, Riddell R, Steinbronn K, Garewal HS. Discordance between flow cytometric abnormalities and dysplasia in Barrett's esophagus. Gastroenterology 1989;97:815-820.

111. Reid BJ, Sanchez CA, Blount PL, Levine DS. Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression. Gastroenterology 1993;105:119-129.

112. Levine DS, Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. Correlation of ultrastructural aberrations with dysplasia and flowcytometric abnormalities in Barrett's epithelium. Gastroenterology 1989;96:355-367.

113. Garewal HS, Sampliner RE, Fennerty MB. Flow cytometry in Barrett's esophagus. What have we learned so far? Dig Dis Sci 1991;36:548-551.

114. Pellish LJ, Hermos JA, Eastwood GL. Cell proliferation in three types of Barrett's epithelium. Gut 1980;21:26-31.

115. Hameeteman W. Cell proliferation study in columnar-lined esophagus. Columnar-lined (Barrett's) esophagus, Chapter 7. Dissertation 1989.

116. Gray MR, Hall PA, Nash J, Ansari B, Lane DP, Kingsnorth AN. Epithelial proliferation in Barrett's esophagus by proliferating cell nuclear antigen immunolocalization. Gastroenterology 1992;103:1769-1776.

117. Jankowski J, McMenemin R, Hopwood D, Fensten J, Wormsley KG. Abnormal expression of growth regulatory factors in Barrett's oesophagus. Clin Sci 1991;81:663-668.

118. Jankowski J, McMenemin R, Yu C, hopwood D, Wormsley KG. Proliferating cell nuclear antigen in oesophageal diseases: correlation with transforming growth factor alpha expression. Gut 1992;33:587-591.

119. Peuchmaur M, Potet F, Goldfain D. Mucin histochemistry of the columnar epithelium of the oesophagus (Barrett's oesophagus): A prospective biopsy study. J Clin Pathol 1984;37:607-610.

120. Lapertosa G, Baracchini P, Fulcheri E. Operative Group for the Study of Esophageal Precancer. Mucin histochemistry in the interpretation of Barrett's esophagus: results of a multicenter study. Am J Clin Pathol 1992;98:61-66.

121. Garewal HS, Sampliner R, Gerner EW, Steinbronn K, Alberts DS, Kendall DS. Ornithine decarboxylase activity in Barrett's esophagus: a potential marker for dysplasia. Gastroenterology 1988;94:819-821.

122. Garewal HS, Sampliner R, Alberts D, Steinbronn K. Increase in ornithine decarboxylase activity associated with development of dysplasia in Barrett's esophagus. Dig Dis Sci 1989;34:312-314.

123. Garewal HS, Gerner EW, Sampliner RE, Roe D. Ornithine decarboxylase and polyamine levels in columnar upper gastrointestinal mucosa in patients with Barrett's esophagus. Cancer Res 1988;3288-3291.

124. Gray MR, Wallace HM, Goulding H, Hoffman J, Kenyon WE, Kingsnorth AN. Mucosal polyamine metabolism in the columnar lined oesophagus. Gut 1993;34:584-587.

125. Geboes K, Vanstapel MJ, Desmet VJ. Tissue demonstration of carcinoembryonic antigen (CEA) in columnar esophageal epithelium. Hepatogastroenterology 1981;28:324-336.

126. Tahara E. Growth factors and oncogenes in human gastrointestinal cancer. J Cancer Res Clin Oncol 1990;116:121-131.

127. Jankowski J, Hopwood D, Wormsley KG. Flow-cytometric analysis of growth-regulatory peptides and their receptors in Barrett's oesophagus and oesophageal adenocarcinoma. Scand J Gastroenterol 1992;27:147-154.

128. Yoshida K, Kyo E, Tsuda T, et al. EGF and TGF alpha, the ligands of hyperproduced EGFR in human esophageal carcinoma cells, act as autocrine growth factors. Int J Cancer 1990;45:131-135.

129. Blount PL, Meltzer SJ, Yin J, Huang Y, Krasna MJ, Reid BJ. Clonal ordering of 17p and 5q allelic losses in Barrett dysplasia and adenocarcinoma. Proc Natl Acad Sci USA 1993;90:3221-3225.

130. Garewal HS, Sampliner R, Liu Y, Trent JM. Chromosomal rearrangement in Barrett's esophagus. A premalignant lesion of esophageal adenocarcinoma. Cancer Genet Cytogenet 1989;42:281-296.

131. Raskind WH, Norwood T, Levine DS, Haggitt RC, Rabinovitch PS, Reid BJ. Persistent clonal areas and clonal expansion in Barrett's esophagus. Cancer Res 1992;52:2946-2950.

132. Rodriguez E, Rao PH, Ladanyi M, et al. 11p13-15 is a specific region of chromosomal rearrangement in gastric and esophageal adenocarcinomas. Cancer Res 1990;50:6410-6416.

133. Huang Y, Boynton RF, Blount PL, et al. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. Cancer Res 1992;52:6525-6530.

134. Blount PL, Galipeau PC, Sanchez CA, et al. 17p Allelic losses in diploid cells of patients with Barrett's esophagus who develop aneuploidy. Cancer Res 1994;54:2292-2295.

135. Blount PL, Ramal S, Raskind WH, et al. 17p Allelic deletions and p53 protein overexpression in Barrett's adenocarcinoma. Cancer Res 1991;51:5482-5486.

136. Casson AG, Mukhopadhyay T, Cleary KR, Ro JY, Levin B, Roth JA. p53 Gene mutations in Barrett's epithelium and esophageal cancer. Cancer Res 1991;51:4495-4499.

137. Boynton RF, Blount PL, Yin J, et al. Proc Natl Acad Sci USA 1992;89:3385-3388.

138. Meltzer SJ, Yin J, Manin B, et al. Microsatellite instability occurs frequently in both diploid and aneuploid cell populations of Barrett's associated esophageal adenocarcinomas. Cancer Res 1994;54:3379-3382.

139. Jeffreys AJ, Wilson V, Neumann R, Keyte J. Amplification of human microsatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. Nucleic Acid Res 1988;16:10953-10971.

140. Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 1992;71:543-546.

141. Livingstone LR, White A, Sprouse J, Livanos E, Jachs Y, Tisty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 1992;70:923-935.

142. Jankowski J, Coghill G, Hopwood D, Wormsley KG. Oncogenes and onco-suppressor gene in adenocarcinoma of the oesophagus. Gut 1992;33:1033-1038.

143. Ramel S, Reid BJ, Sanchez CA, et al. Evaluation of p53 protein expression in Barrett's esophagus by two-parameter flow cytometry. Gastroenterology 1992;102:1220-1228.

144. Yonnes M, Lebovitz RM, Lechago LV, Lechago J. p53 Protein accumulation in Barrett's metaplasia, dysplasia and carcinoma: a follow-up study. Gastroenterology 1993;105:1637-1642.

145. Flejou JF, Potet F, Muzeau F, Le Pelletier F, Fekete F, Henin D. Overexpression of p53 protein in Barrett's syndrome with malignant transformation. J Clin Pathol 1993;46:330-333.

146. Hardwick RH, Shepherd NA, Moorghen M, Newcomb PV, Alderson D. Adenocarcinoma arising in Barrett's oesophagus: evidence for the participation of p53 dysfunction in the dysplasia/carcinoma sequence. Gut 1994;35:764-768.

147. Stemmerman G, Heffelfinger SC, Noffsinger A, Hui YZ, Miller MA, Fenoglio-Preiser CM. The molecular biology of esophageal and gastric cancer and their precursors: oncogenes, tumor suppressor genes, and growth factors. Human Pathol 1994;25:968-981.

148. Houldsworth J, Cordon-Cardo C, Ladanyi M, Kelsen DP, Chaganti RSK. Gene amplification in gastric and esophageal adenocarcinomas. Cancer Res 1990;50:6417-6422.

149. Al-Kasspooles M, Moore JH, Orringer MB, et al. Amplification and overexpression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer 1993;54:213-219.

150. Abdelatif OMA, Chandler FW, Mills LR, et al. Differential expression of c-myc and H-ras oncogenes in Barrett's epithelium; a study using colorimetric in situ hybridization. Arch Pathol Lab Med 1991;115:880-885.

151. Meltzer SJ, Zhou D, Weinstein WM. Tissue-specific expression of c-HA-ras in premalignant gastrointestinal mucosae. Experimental and Molecular Pathol 1989;51:264-274.

152. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. Gastroenterology 1993;104:510-513.

153. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287-1289.

154. Fein R, Kelsen DP, Geller N, Bains M, McCormack P, Brennan MF. Adenocarcinoma of the esophagus and gastroesophageal junction: Prognostic factors and results of therapy. Cancer 1985;56:2512-2518.

155. DeMeester TR. Barrett's esophagus. Surgery 1993;113:239-241.

156. Bremner RM, DeMeester TR. Surgical treatment of esophageal carcinoma. Gastroenterol Clin N Am 1991;20:743-763.

157. Bosch A, Frias Z, Calswell WL. Adenocarcinoma of the esophagus. Cancer 1979;43:1557-1561.

158. Skinner DB, Ferguson MK, Soriano A, Little AG, Staszak VM. Selection of operation for esophageal cancer based on staging. Ann Surg 1986;204:391-400.

159. Powell J, McConkey CC. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. Br J Cancer 1990;59:440-443.

160. Haggitt RC, Trijzelaar J, Ellis FH, Colcher H. Adenocarcinoma complicating columnar epitehlium (Barrett's) esophagus. Am J Clin Pathol 1978;70:1-5.

161. Schnell TG, Sontag SJ, Chejfec G. Adenocarcinomas arising in tongues or short segments of Barrett's esophagus. Dig Dis Sci 1992;37:137-143.

162. Ovaska J, Miettinen M, Kivilaakso E. Adenocarcinoma arising in Barrett's esophagus. Dig Dis Sci 1989;34:1336-1339.

163. Atkinson m. Barrett's oesophagus - to screen or not to screen? Gut 1989;30:2-5.

164. Sanfey H, Hamilton SR, Smith RRL. Carcinoma arising in Barrett's esophagus. Surg, Gyn & Obstet 1985;161;570-574.

165. Levine MS, Caroline D, Thompson JJ, Laufer I, Herlinger H. Adenocarcinoma of the esophagus: relationship to Barrett's mucosa. Radiology 1984;150:305-309.

166. Hameeteman W. The endoscopic view of columnar-li esophagus and its complications. Columnar-lined (Barrett's) esophagus, Chapter 2. Thesis, 1989.

167. Geisinger KR, Teot LA, Richter JE. A comparative cytopathologic and histologic study of atypia, dysplasia and adenocarcinoma in Barrett's esophagus. Cancer 1992;69:8-16.

168. Wang HH, Doria MI Jr., Purohit-Buch S, Schnell T, Sontag S, Chejfec G. Barrett's esophagus. The cytology of dysplasia in comparison to benign and malignant lesions. Acta Cytologica 1992;36:60-64.

169. Ohno S, Mori M, Tsutsui S, Matsuura H, Kuwano H, Soejima K, Sugimachi K. Growth patterns and prognosis of submucosal carcinoma of the esophagus. A pathologic study. Cancer 1991;68:335-340.

170. Pascal RR, Clearfield HR. Mucoepidermoid (adenosquamous) carcinoma arising in Barrett's esophagus. Dig Dis Sci 1987;32:428-432.

171. Rosengard A, Hamilton SR. Squamous carcinoma of the esophagus with Barrett's esophagus. Mod pathol 1989;2:2-7.

172. Paraf F, Flejou JF, Potet F, Molas G, Fekete F. Esophageal squamous carcinoma in five patients with Barrett's esophagus. Am J Gastroenterol 1992;87:746-750.

173. Siewert JR, Holscher AH, Dittler HJ. Preoperative staging and risk analysis in esophageal carcinoma. Hepatogastroenterol 190;37:382-387.

174. Becker CD, Barbier P, Porcellini B. CT evaluation of patients undergoing transhiatal esophagectomy for cancer. J Comput Assist Tomogr 1986;10:607-611.

175. van Overhagen H, Lameris JS, Berger MY, Klooswijk AIJ, Tilanus HW, van Pel R, Schutte HE. CT assessment of resectability prior to transhiatal esophagectomy for esophageal/gastroesophageal junction carcinoma. J Comput Assist Tomogr 1993;17:367-373.

176. Ziegler K, Sanft C, Zeitz M, et al. Evaluation of endosonography in TN-staging of esophageal cancer. Gut 1991;32:16-20.

177. Tio TL, Cohen P, Coene PP, et al. Endosonography and computed tomography of esophageal carcinoma. gastroenterology 1989;96:1478.

178. Toh Y, Baba K, Ihebe M, Adachi Y, Kuwano H, Sugimachi K. Endoscopic ultrasonography in the diagnosis of an early esophageal carcinoma. Hepatogastroenterol 1993;40:212-216.

179. Quint LE, Glazer GM, Orringer MB. Esophageal imaging by MR and CT: study of normal anatomy and neoplasms. Radiology 1985;156:727.

180. Lehr L, Rupp N, Siewert JR. Assessment of resectability of esophageal cancer by computed tomography and magnetic resonance imaging. Surgery 1988;103:344-350.

181. van Overhagen H, Lameris JS, Berger MY, Tilanus HW, van Pel R, Klooswijk AIJ, Schutte HE. Improved assessment of supraclavicular and abdominal metastases in oesopahgeal and gastro-oesophageal junction carcinoma with the combination of ultrasound and computed tomography. Br J Radiol 1993;66:203-208.

182. van Overhagen H, Lameris JS, Berger MY, van Pel R, TILANUS HW, Klooswijk AIJ, Schutte HE. Assessment of distant metastases with ultrasound-guided fine-needle aspiration biopsy and cytologic study in carcinoma of the esophagus and gastroesophageal junction. Gastrointest Radiol 1992;17:305-310.

183. Molloy RG, McCourtney JS, Anderson JR. Laparoscopy in the management of patients with cancer of the gastric cardia and oesophagus. Br J Surg 1995;82:352-354.

184. Siewert JR, Holscher AH, Bollschweiler E. Surgical therapy of cancer in Barrett's esophagus. Diseases of the esophagus 1992;5;57-62.

185. Orringer MB. Transhiatal esophagectomy without thoracotomy for carcinoma of the thoracic esophagus. Ann Surg 1984;200;282-288.

186. Lerut T, Coosemans W, Van Raemsdonck D, Dillemans B, De Leyn P, Marnette JM. Surgical treatment of Barrett's carcinoma. Correlation between morphologic findings and prognosis. J Thorac Cardiovasc Surg 1994;107;1059-1066.

187. Plukker JTh, Mulder NH, Sleyfer DTh, Grond J, verschueren RCJ. Chemotherapy and surgery for locally advanced cancer of the cardia and fundus: phase II study with methotrexate and 5-fluorouracil. Br J surg 1991;78;955-958.

188. Gignoux M, Roussel A, Paillot B, et al. the value of preoperative radiotherapy in esophageal cancer; results of a study of the EORTC. World J Surg 1987;11:426-432.

189. Rowland CG, Pagliero KM. Intracavitary irradiation in palliation of oesophagus and cardia. Lancet 1985;ii:981-983.

190. Krasner N, Barr H, Skidmore C, Morris AL. Palliative lasertherapy for malignant dysplasia. Gut 1987;28;792-798.

191. Johnsten JH, Fleisher D, Petrini J, Norel HJ. Palliative bipolar electrocoagulation therapy of obstructing esophageal cancer. Gastrointest Endosc 1987;33:349-353.

192. Tytgat GNJ, Hartog Jager FCA den, Bartelsman JFWM. Endoscopic prothesis for advanced esophageal cancer. Endoscopy 1986;18:32-39.

193. Nishimaki T, Tanaka O, Suzuki T, Aizawa k, watanabe H, Muto T. Tumor spread in superficial esophageal cancer: histopathologic basis for rational surgical treatment. World J Surg 1993;17:766-772.

194. UICC (International Union Against Cancer). TNM Classification of malignant tumours. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer-Verlag, 1987.

195. Kato H, Tachimore Y, Watanabe H, Yamaguchi H, Ishikawa T, itabashi M. Superficial esophageal carcinoma. surgical treatment and results. Cancer 1990;66:2319-2323.

196. Lerut T, De Leyn P, Coosemans W, Van Raemdonck D, Scheijs I, Lesaffre E. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. Ann Surg 1992;216:583-590.

CHAPTER 2

Prognostic factors for the survival of patients with an adenocarcinoma in Barrett's oesophagus

CHAPTER 2.1

Outcome of surgical treatment of adenocarcinoma in Barrett's oesophagus

MBE Menke-Pluymers, NW Schoute, AH Mulder, WCJ Hop, M van Blankenstein, HW Tilanus

Published in:
Gut 1992; 33: 1454-1458.

Summary

To analyse the survival of 112 patients (85 men and 27 women, mean age 63 years) with an adenocarcinoma in a columnar lined oesophagus in relation to surgical treatment, tumour staging and histological grading, a retrospective study was performed over an 11-year period (1978-1988).

Presenting symptoms were dysphagia (60%) and pain (25%). Only 6 patients were previously known to have a columnar lined oesophagus. Eighty-five patients (76%) underwent a partial resection of the oesophagus and cardia. Postoperative mortality was 6%. After resection (N=85) the 5-year survival was 24%. Survival was significantly better for patients without regional lymph node metastases (Stage 0,I,IIA (N=61): 5-year survival 30%) and even better if the tumour was restricted to the submucosa (Stage 0,I (N=12): 5-year survival 63%). Survival was not influenced by the histological grade of the tumour.

In conclusion, staging for adenocarcinoma in columnar lined oesophagus based on infiltration of the oesophageal wall and lymph node spread is valuable in determining the prognosis.

Introduction

Barrett's oesophagus (1,2) represents a metaplastic transformation of the normal squamous cell epithelium of the lower tubular oesophagus into columnar epithelium (3). The premalignant character of Barrett's oesophagus is demonstrated by progression from benign columnar epithelium into dysplasia and adenocarcinoma (4,5). Although endoscopic surveillance and biopsy are performed to achieve an early diagnosis of malignant degeneration, many patients with Barrett's oesophagus present primarily with symptoms of an oesophageal carcinoma.

The survival of patients with an adenocarcinoma in Barrett's oesophagus is low, even after oesophagectomy (6,7). The influence of infiltration of the oesophageal wall, lymph node metastases and histological grade of the tumour on the prognosis is not fully understood. The purpose of this retrospective study was to analyse the survival of patients with an adenocarcinoma in columnar lined oesophagus in relation to surgical treatment, staging and differentiation grade of the tumour.

Patients and methods

During the period 1978-1988, 112 consecutive patients with an adenocarcinoma in a columnar lined lower oesophagus were referred to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment. The patient population consisted of 85 men (76%) and 27 women (24%), with a mean age of 63 years (range 30-96 years).

Barrett's columnar lined oesophagus is defined as the condition in which columnar type epithelium is found at least 3 centimeters above the distal end of the oesophagus as endoscopically defined. The demarkation between stomach and oesophagus is endoscopically determined by the diameter change of the lumen, change in colour of the mucosa and vascular pattern. The upper level was diagnosed by the columnar-squamous epithelial border, which was always easily identified. For the purpose of this study, we considered those patients with a biopsy proven adenocarcinoma of the distal tubular oesophagus, in whom columnar epithelium of the oesophagus above the carcinoma was visible macroscopically at endoscopy, meeting the above definition of Barrett's

oesophagus. Patients with a carcinoma of the cardia were excluded from this study.

A careful history covering the total livespan with respect to symptoms of reflux and anti-reflux treatment was obtained. Preoperatively all patients underwent a general physical examination, electrocardiogram and lung function studies to determine their general fitness for operation. Chest X-ray, barium swallow studies, endoscopy with biopsy, ultrasound examination of the liver and upper abdominal areas, and in cases of high- or mid-oesophageal tumours bronchoscopy were performed. Since 1980 computer tomography of the thorax and upper abdomen and ultrasound examination of the neck were carried out in order to identify pathological lymph nodes, which were subjected to cytological examination (8). All patients considered inoperable on grounds of general health, with distant metastases or extension of the tumour into the bronchus were not considered for resection.

During the period 1978-1985 all patients who were considered for resection (N=72) received preoperative radiation therapy (40 Gray in 4 weeks). However, in two randomized prospective studies no advantage of preoperative radiation therapy could be demonstrated (9,10). Therefore, from 1986-1988 preoperative radiation therapy was no longer given (N=27). Hospital mortality was defined as the mortality during postoperative stay in hospital.

Based on pathologic analysis of the resection specimens, postoperative staging was performed according to the pTNM-classification of the U.I.C.C., 1987(11) (table 1). At least half of the tumour was examined histologically. Those areas which were suspect for deep invasive growth were selected. Areas immediately above the tumour were also investigated for the presence of metaplastic epithelium. Biopsies and resection specimens were reviewed for the differentiation grade of the tumour.

The follow-up period ranged from 1 month to 9 years with a mean of 21 months. Survival was assessed by computerised life table analysis according to Kaplan-Meier (12). For all patients survival after diagnosis was calculated. For comparison of the survival of subgroups of patients, the Logrank test was used. Other methods used are indicated in the text. $P<0.05$ (two-sided) was considered the limit of statistical significance.

Table 1. P-TNM-classification of oesophageal carcinoma according to the U.I.C.C., 1987.

Stage grouping				Number of patients
Stage 0 :	Tis	N0	M0	2
Stage I :	T1	N0	M0	10
Stage IIA:	T2	N0	M0	19
	T3	N0	M0	30
Stage IIB:	T1	N1	M0	1
	T2	N1	M0	4
Stage III:	T3	N1	M0	13
	T4	anyN	M0	2
Stage IV :	anyT	anyN	M1	4

Tis (carcinoma in situ), T1 (tumour invades lamina propria or submucosa), T2 (tumour invades muscularis propria), T3 (tumour invades adventitia), T4 (tumour invades adjacent structures), N0 (no regional lymph node metastases), N1 (regional lymph node metastases), M0 (no distant metastases), M1 (distant metastases).

Results

Symptoms and social habits.

The most important presenting symptoms were dysphagia (60%) and pain (25%). Patient characteristics with respect to symptoms of gastro-oesophageal reflux and treatment are listed in Table 2. Twelve patients (11%) had undergone an antireflux operation in the past, with an interval between anti-reflux surgery and diagnosis of an oesophageal carcinoma ranging from two months to 33 years (median 4.75 years, mean 10 years). In three patients a Nissen fundoplication had been performed, a Billroth II partial gastric resection in three, a high selective vagotomy in one, and in five patients the procedure performed for hiatal hernia was not precisely documented. Daily alcohol consumption was reported by 67% of the patients, with more than 4 consumptions a day by 4%. Fifty-one percent of the patients were cigarette smokers.

Endoscopic findings.

Of all 112 patients with an adenocarcinoma, only 6 (5%) had a previous biopsy proven history of columnar lined oesophagus. The area of adenocarcinoma was circular in 41 patients (36%), and comprised more than half the circumference of the oesophagus in 19 patients (17%). On average the tumour extended over a length of 5 cm of the distal tubular oesophagus (ranging from 1-12 cm). In the group of patients with a resectable tumour (n=85) the mean length (± SD) of the tumour was 4 ± 2 cm, and in the group with an irresectable tumour (n=27) 6 ± 2 cm (Mann-Whitney's test: p=0.003). The columnar lined segment comprised 3-19 cm (mean 6 cm) of the oesophagus above the tumour. A hiatal hernia was found in 72% of the patients on endoscopical examination.

Table 2. Symptoms of gastro-oesophageal reflux and treatment in patients with adenocarcinoma in columnar-lined oesophagus.

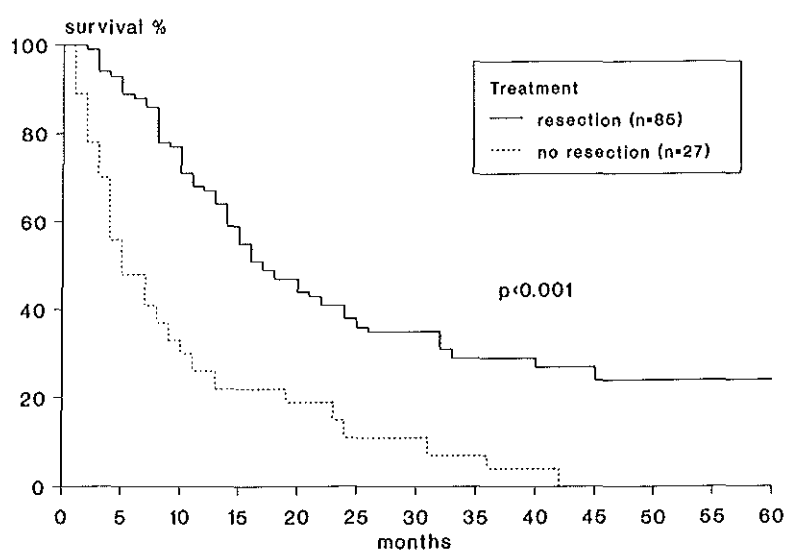
Symptoms	Patients N (%)	Anti-reflux medication (N)	Anti-reflux surgery (N)
No symptoms	39 (35)		
Heartburn/ Regurgitation	62 (55)	33	12
- < 1 year	10 (9)	4	2
- 1-5 years	9 (8)	5	1
- > 5 year	32 (28)	19	6
- unknown duration	11 (10)	5	3
Not documented	11 (10)		

Treatment.

After preoperative screening, 99 patients (88%) with an adenocarcinoma in columnar lined oesophagus were considered for surgical treatment. In 14 patients an irresectable tumour or metastases were found at laparotomy. Eighty-five patients (76%) underwent a subtotal resection of the oesophagus and cardia. Reconstruction was made by

gastric tube (N=79) or colonic interposition (N=6), with an intrathoracic anastomosis at the level of the azygos vein or a cervical anastomosis. Postoperative complications occurred in 29 patients (morbidity rate 34%), of which the most important were postoperative bleeding (n=6), recurrent laryngeal nerve paresis (n=6) and anastomotic leakage (n=8). Cardiovascular complications in three, respiratory complications in two and postoperative bleeding in one patient lead to death in six patients during the postoperative period in hospital (hospital mortality 6%). Of the 14 irresectable patients no one died in hospital after operation. Over the first period of study (1978-1983) hospital mortality was 9%, while over the second period (1984-1988) it had reduced to 4% (Fisher exact test: p=0.4). Sixty-six of the 85 resected patients (78%) had received radiation therapy (40 Gray in 4 weeks) prior to operation, as a routine preoperative treatment until 1986. The 27 inoperable or irresectable patients were treated by non-surgical treatment with palliative intent.

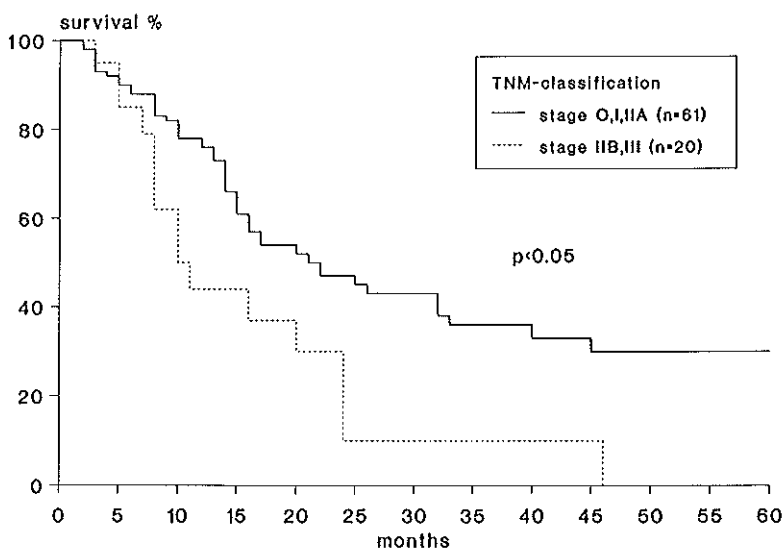
Figure 1. Survival (all causes) of patients treated by oesophagectomy (n=85) versus inoperable or irresectable patients (n=27).



Pathologic findings and staging.

Reviewing the slides for the differentiation grade of the tumour, in 2 cases the tumour was classified as well differentiated, in 52 cases as moderately and in 58 cases as poorly differentiated. In 95% of the cases the margins of the resected specimens were free of tumour tissue on histological examination, but in 4 cases (5%) microscopical remnants were demonstrated. Patients were classified according to the pTNM-classification as shown in Table 1. Barrett's mucosa was histologically proven in all cases.

Figure 2. Survival of resected patients without regional lymph node metastases (n=61) versus patients with regional lymph node metastases (n=20).



Survival.

At the end of the study 26 patients were alive, 6-107 months (mean 36 months) after resection of the tumour. Sixty-two patients died from metastatic disease (72% of all deaths), 18 from causes unrelated to carcinoma and in 6 cases the cause of death was unknown. Life table analysis revealed 1-, 2- and 5-year survival rates of respectively 57%, 31% and 17% for the whole group. The 1-, 2- and 5-year survival rates of the 85 patients who underwent oesophagectomy were respectively 67%, 38% and 24%, and for

the 27 inoperable or irresectable patients respectively 26%, 11% and 0% ($p<0.001$), Figure 1. Within the group of resected patients (N=85) were no significant differences in survival between patients treated by preoperative radiation therapy (N=66) and those treated by resection alone (N=19). Patients classified as stage 0, I or IIA (no regional lymph node metastases) had a significant better survival ($p=0.03$) with 1-, 2- and 5-year survival rates of respectively 76%, 47% and 30%, as compared to patients classified as stage IIB and III (regional lymph node metastases present), with 1-, 2- and 5-year survival rates of respectively 44%, 10% and $\leq 10\%$ (Figure 2). Patients with a Tis or T1 tumour without regional lymph nodes (N=12) had a significant better survival than patients with a T2 or T3 tumour without regional lymph nodes (N=49), $p<0.05$ (Figure 3). Patients with well (GI) or moderately (GII) differentiated tumours classified as stage 0, I, IIA and IIB, III respectively did not show a significant better survival than patients with poorly differentiated (GIII) tumours (Figure 4).

Figure 3. Survival of resected patients classified as stage 0 or I (n=12) versus patients classified as stage IIA (n=49).

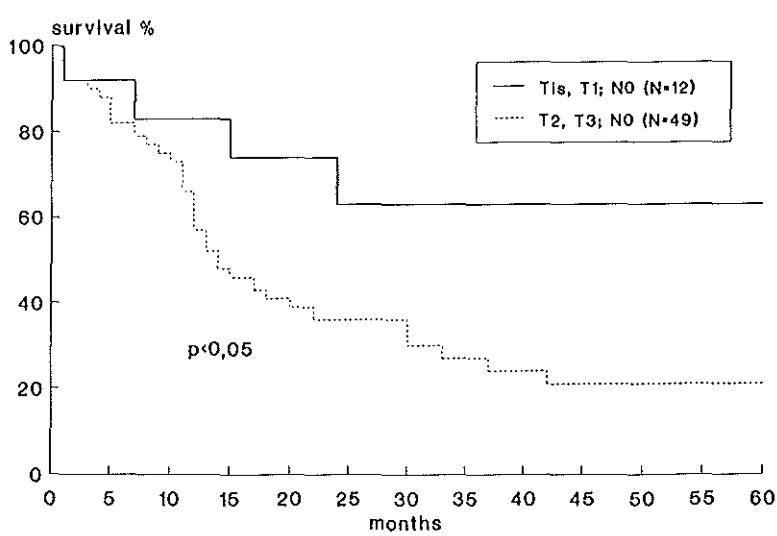
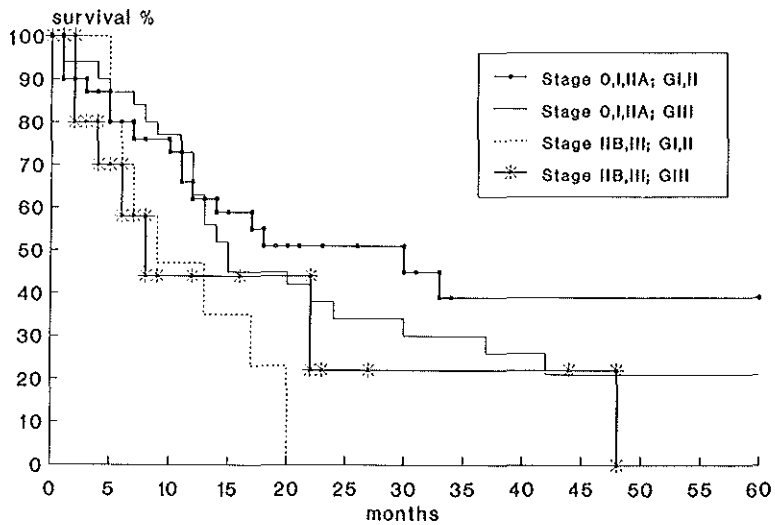


Figure 4. Survival of resected patients with well or moderately differentiated tumours (GI,II) versus patients with poorly differentiated tumours (GIII) staged respectively as stage 0,I,IIA and stage IIB,III.



Discussion

In 1950 Barrett described the condition of columnar epithelium lining a mediastinal stomach, secondary to a congenital short oesophagus (1). Allison and Johnstone suggested that columnar epithelium occurred in the lower oesophagus, secondary to gastro-oesophageal reflux (3). Since Barrett's description of the condition in the early nineteen fifties, many investigators have noted an association between columnar lined oesophagus and primary oesophageal carcinoma (2,4-7).

The reported mean age of patients with an adenocarcinoma associated with Barrett's oesophagus is about 57 years and the male to female ratio 5.5:1 (14). In our series, the mean age was higher (63 years), and the male to female ratio lower (3.1:1) than reported, but still indicating a male predominance. In our patient group a major presenting symptom was dysphagia, which is in agreement with the presenting symptoms reported in the literature (7).

Controversy exists regarding the influence of medical or surgical treatment of gastro-oesophageal reflux on Barrett's oesophagus. Medical treatment (antacids, H2-receptor blocking agents, prokinetic drugs) has no influence on the extent of Barrett's mucosa (14), but surgical correction of gastro-oesophageal reflux leads possibly to partial regression of Barrett's epithelium (15,16). However, 14 patients in our series had undergone antireflux surgery in the past. This suggests that an antireflux operation will not inevitably lead to regression or disappearance of Barrett's epithelium, and may not diminish the risk of malignant degeneration.

The treatment of choice most often reported for adenocarcinoma of the oesophagus is surgical (7,13,19). The reported postoperative mortality after oesophagectomy for carcinoma varies between 10-16% (7,17,18). However, most of these studies comprised patient populations treated by varying surgical techniques, in a period before our study. While most patients with an adenocarcinoma associated with Barrett's oesophagus are resectable, the long term survival is low (7). In our series the 5-year o2verall survival was 17% and after resection and reconstruction 24%, which is in concordance with the reported 5-year survival (14.5-22%) (7,19).

Seventy-two percent of the resected patients did not have lymph node involvement, and more than one third (36%) had neither node involvement nor full thickness wall penetration of the oesophagus, which seems favourable regarding the literature (7,17). A clear correlation between pathologic staging of oesophageal tumours and prognosis has been reported, particularly infiltration through the muscular wall and regional lymph node metastases have a negative influence on survival rates (17,20,21). Within the group of resected patients in our series, those without regional lymph node metastases showed a significant better survival compared to patients with regional lymph node metastases. Survival was significantly better if the tumour was restricted to the submucosa if compared to those cases in which the tumour invaded the muscle layer.

The influence of histological differentiation grade of adenocarcinomas in Barrett's oesophagus has not been reported before. For squamous cell carcinomas of the oesophagus histological differentiation grade has no significant effect on survival (20). However, in one large series including all types of oesophageal carcinomas patients late outcome was influenced significantly by tumour staging and histological grading (21). In our series histological differentiation grade of the tumour had no significant influence on survival.

Conclusion.

Most patients with Barrett's oesophagus were not recognized prior to the diagnosis of an associated adenocarcinoma. If an adenocarcinoma in Barrett's oesophagus is diagnosed, resection offers the only chance for cure. In our series 5-year survival after resection was 24%. A correlation between pathologic staging and survival was demonstrated, with a significant better survival if the tumour was restricted to the submucosa and in absence of regional lymph node metastases. Survival was not influenced by histological differentiation grade op the tumour.

References

1. Barrett NR. Chronic peptic ulcer of the oesophagus and oesophagitis. Br J Surg 1950; 38:175-82.
2. Barrett NR. The lower oesophagus lined by columnar epithelium. Surgery 1957; 41: 881-94.
3. Allison PR, Johnstone AS. The oesophagus lined with gastric mucous membrane. Thorax 1953; 8: 87-101.
4. Naef AP, Savary M, Ozzello L. Columnar-lined lower esophagus: an acquired lesion with malignant potential. J Thorac Vasc Surg 1975; 70: 826-35.
5. Hameeteman W, Tytgat GNJ, Houthoff HJ, Van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989; 96: 1249-56.
6. Starnes VA, Adkins RB, Ballinger JF, Sawyers JL. Barrett's esophagus. A surgical entity. Arch Surg 1984; 119: 563-67.
7. Sanfey H, Hamilton SR, Smith RRL, Cameron JL. Carcinoma arising in Barrett's oesophagus. Surg Gynecol Obstet 1985; 161: 570-74.
8. Overhagen van H, Lameris JS, Berger MY et al. Supraclavicular lymph node metastases in carcinoma of the esophagus and gastro-esophageal junction: assessment with CT, US, and US guided fine-needle aspiration biopsy. Radiology 1991; 79: 155-158.
9. Launois B, Delarue D, Campion JP, Kerbaol M. Preoperative radiotherapy for carcinoma of the esophagus. Surg Gynecol Obstet 1981; 153: 690-92.
10. Gignoux M, Roussel A, Paillot B, et al. The value of preoperative radiotherapy in esophageal cancer: results of a study of the EORTC. World J Surg 1987; 11: 426-32.
11. UICC (International Union Against Cancer). TNM Classification of malignant tumours. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer-Verlag, 1987.
12. Kaplan EL, Meier EA. Nonparametric estimation from incomplete observations. J Am Statist Assoc 1958; 53: 457-81.

13. Bremner CG. The management of columnar-lined oesophagus. In: Surgery of the oesophagus, Jamieson GG (ed.), Churchill Livingstone 1988.

14. Sampliner RE, Garewal HS, Fennerty MB, Aickin M. Lack of impact of therapy on extant of Barrett's esophagus in 67 patients. Dig Dis Sci 1990; 35: 93-6.

15. Brand DL, Ylvisaker JT, Gelfand M, Pope CE. Regression of columnar esophageal (Barrett's) epithelium after antireflux surgery. N Engl J Med 1980; 302: 884-48.

16. Williamson WA, Ellis FH Jr., Gibb SP, Shahian DM, Aretz HT. Effect of antireflux operation on Barrett's mucosa. Ann Thorac Surg 1990; 49: 537-41.

17. Skinner DB, Ferguson MK, Soriano A, Little AG, Staszak VM. Selection of operation for oesophageal cancer based on staging. Ann Surg 1986; 204: 391-401.

18. Hennessy TPJ, O'Connell R. Surgical treatment of squamous cell carcinoma of the oesophagus. Br J Surg 1984; 71: 750-51.

19. Skinner DB, Walther BC, Riddell RH, Schmidt H, Iascone C, DeMeester T. Barrett's esophagus: Comparison of benign and malignant cases. Ann Surg 1983; 198: 554-56.

20. Hambraeus GM, Mercke CE, Willen R, Ranstam J, Samuelsson L, Lamm IL. Prognostic factors influencing survival in combined radiotherapy and surgery of squamous cell carcinoma of the esophagus with special reference to a histopathological grading system. cancer 1988; 62(5): 895-904.

21. Muller JM, Jarczyk AJ, Huber P, Pichlmaier H. Ergebnisse der resektion der Speiserohre wegen eines carcinoms. Chirurg 1988; 59(69): 398-406.

CHAPTER 2.2

DNA-ploidy as a prognostic factor for patients with an adenocarcinoma in Barrett's oesophagus

MBE Menke-Pluymers, WCJ Hop, AH Mulder, HW Tilanus

Hepato-Gastroenterology, in press.

Summary

The role of DNA-ploidy of tumour cells as a prognostic factor for the survival after resection was evaluated in 40 patients with an adenocarcinoma in Barrett's oesophagus (28 men and 12 women with a mean age of 61 years).

After resection of the oesophagus staging was performed according to the TNM-classification (UICC 1987). Tumour tissue specimens were reviewed for the histological differentiation grade and DNA-flowcytometry was performed on paraffin embedded tissue according to the method described by Hedley.

There was no significant correlation between DNA-ploidy and TNM-stage or histological differentiation grade of the tumour. DNA ploidy, stage and grade as prognostic factors for the survival were evaluated by multivariate analysis. DNA ploidy significantly ($p=0.04$) correlated with survival, also univariately. Patients with diploid tumours had 3- and 5-year survival rates of 51% and 25% respectively, as compared to 10% and 0% respectively for patients with aneuploid or tetraploid/increased G2 tumours.

Although the number of patients in this study is relatively small, there is an indication that DNA-ploidy may be a prognostic factor for the survival of patients with an adenocarcinoma in Barrett's oesophagus.

Introduction

Patients with a carcinoma of the oesophagus have a dismal prognosis, even after resection. We previously reported the survival of 112 patients with an adenocarcinoma in Barrett's oesophagus referred to the Rotterdam Oesophageal Tumour Study Group during the period 1978-1988 (1). The 5-year survival after oesophagectomy was 24%. The most important prognostic factors for the survival of patients after resection are the extent of tumour growth in the oesophageal wall and the presence of regional lymph node metastases (1-3).

Human malignancies develop in association with a process of genomic instability, and many human solid tumours show abnormalities of the cellular DNA content (4-9). There is evidence of a relation between DNA-ploidy pattern and prognosis of a variety of malignant tumours (4-9). Flowcytometry of formalin fixed paraffin embedded tissue allows retrospective study of archival material of patients whose clinical outcome is already known (10).

To evaluate the role of DNA-ploidy of the tumour cells as a prognostic factor for the survival, we analyzed paraffin embedded tumour tissue specimens of patients with an adenocarcinoma in Barrett's oesophagus referred to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment between 1978-1988.

Patients and methods

Patients.

During the period 1978-1988, 112 patients (85 men, 27 women; mean age, 63 years) with an adenocarcinoma in Barrett's oesophagus were referred to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment. Eighty-five patients (76%) underwent a subtotal resection of the oesophagus and cardia. As a routine preoperative treatment until 1986, 66 of the 85 (78%) resected patients had received radiation therapy (40 Grays over four weeks) before operation. Tumour samples of resection specimens from those patients not receiving preoperative radiation therapy, as well as endoscopically directed biopsies obtained before radiation therapy was given, were histologically reexamined by light microscopy for the differentiation grade of the tumour. A representative specimen containing adenocarcinoma

was selected for flowcytometry. In 40 cases, paraffin embedded tumour tissue was available for flowcytometry. This group of 40 patients consisted of 28 men (70%) and 12 women (30%) with a mean age of 61 years (range 39-78 years). The tumours were staged according to the TNM-classification (UICC 1987) (11). The follow-up period ranged from one month to 9 years after resection of the oesophagus (mean 2 years).

Specimens

All adenocarcinoma specimens were obtained either with endoscopically directed biopsies or from resection specimens fixed in formalin and embedded in paraffin. These specimens were prepared for flowcytometry according to the method described by Hedley (10). The nuclear suspension was filtered through a 40 micrometer mesh filter and stained with ethidium bromide (BDH Chemicals Ltd. Pook England). Cellular DNA-content of at least 10,000 cells was measured on a fluorescence activated cell sorter (FACS II Becton Dickinson, Sunnyvale California). The Argon laser was tuned at 488 nm and a red fluorescence was measured using a cut off filter (Pomfrett Stamford, Cann.). The data were fed into a Data General computer.

Flowcytometry

Aneuploidy was determined both by visual inspection of the histograms and confirmation of an aneuploid population by calculating the deoxyribonucleic acid index as defined by the Convention of Nomenclature for deoxyribonucleic acid cytometry (12). A diploid histogram contained a G0/G1 peak (DNA-index 1.00) and a G2/tetraploid peak above DNA-index 1.90 and below DNA-index 2.10, with a G2/tetraploid fracion up to 10%. G2/tetraploid fractions above 10% were considered abnormal. Specimens were excluded from analysis if the coefficient of variation exceeded 9%.

Statistics

Survival was assessed by the Kaplan-Meier method. Stage, grade and ploidy as prognostic factors for the survival were evaluated using Cox regression (13). $P=0.05$ (two-sided) was considered the limit of statistical significance.

Results

The results of flowcytometry according to TNM-stage and differentiation grade of the tumour are summarized in table 1. Ploidy did not correlate significantly with TNM-stage or differentiation grade of the tumour. After excluding the 5 patients with histograms with high coefficients of variation (HCV, CV > 9%) and the only patient with distant metastases (stage IV), life table analysis was performed for the remaining 34 patients.

Table 1. Results of flowcytometry of adenocarcinoma in Barrett's oesophagus according to TNM-stage and differentiation grade.

	Diploid (N)	Aneuploid or increased tetraploid/G2 (N)	HCV (N)	Total (N)
TNM-stage				
stage 0	0	1	1	2
stage I	1	1	2	4
stage IIA	6	15	1	22
stage IIB	1	-	-	1
stage III	2	7	1	10
stage IV	1	-	-	1
Grade				
well	1	1	-	2
moderate	5	12	2	20
poor	5	11	3	18

HCV = High Coefficient of Variance (>9%)

Table 2 gives the outcomes of the multivariate analysis of the factors lymph node metastases, differentiation grade and DNA ploidy. Although patients with poorly differentiated tumours did worse as compared to those with well or moderately differentiated

tumours, the difference was not significant. The same applied to the comparison of N+ (with lymph node metastases) to N0 (without lymph node metastases) patients. DNA ploidy significantly (p=0.04) correlated with survival, also univariately. The 3- and 5-year survival percentages in the group of patients with diploid tumours were respectively 51% and 25%. The corresponding percentages in the group with aneuploid or tetraploid/increased G2 tumours were respectively 10% and 0%.

Due to the relatively small number of patients the confidence limits of the relative death rates are rather wide. Therefore conclusions about the prognostic impact from this series are precluded.

Table 2. Outcomes of multivariate analysis of the prognostic factors TNM-stage, differentiation grade and DNA-ploidy for the survival of patients with an adenocarcinoma in Barrett's oesophagus after resection.

Factor	Relative Death Rate	Significance (p-value)	95% confidence limits
Stage			
- N0 (n=24)	1*	-	-
- N+ (n=10)	1.5	0.44	(0.5 , 4.5)
Grade			
- well/moderate (n=20)	1*	-	-
- poor (n=14)	2.2	0.11	(0.8 , 6.1)
Ploidy			
- diploid (n=10)	1*	-	-
- aneuploid or increased tetraploid/G2 (n=24)	3.5#	0.04	(1.1 , 11.5)

* reference category

\# relative death rate is 2.9 (p=0.04) when the factor ploidy was evaluated univariately.

Discussion

Overall 5-year survival after oesophagectomy for adenocarcinoma in Barrett's oesophagus is 24% (1,3). However, survival is influenced by tumour stage, especially with respect to the presence of lymph node metastases (1-3). In this study survival was better for patients without lymph node metastases (3-year survival 25%) compared to those with lymph node metastases (3-year survival 0%), but was not statistically different. This is most likely due to the relatively small number of patients in this study. Histological differentiation grade of the tumour does not seem to play an important role as prognostic factor for the survival of patients with an oesophageal carcinoma (1,14).

Sixty percent of the adenocarcinomas in Barrett's oesophagus had aneuploid or increased G2/tetraploid cell populations. Of squamous cell carcinomas of the oesophagus, 49% were found to be aneuploid in early carcinoma and 83% in more advanced stages (4,5). We found no significant relation between DNA-ploidy and TNM-stage or differentiation grade of the tumour. This agrees with other studies on human solid tumours in which DNA-ploidy was not often found to correlate with clinical stage or histological differentiation grade (6-8).

It has been suggested that aneuploid cancers are generally associated with inferior prognoses compared to diploid ones. A relationship between DNA-ploidy and survival of patients with solid tumours has been reported for several organs (4-9). Matsuura et al. found that the DNA distribution pattern was a major independent determinant of survival in patients with advanced squamous cell carcinoma of the oesophagus (4). Ikebe et al. also found a good correlation between DNA distibution pattern of early squamous cell carcinoma and prognosis (8). Among patients with an adenocarcinoma in Barrett's oesophagus we found a better survival for patients with diploid tumours as compared to those with aneuploid or increased G2/tetraploid tumours.

Although the number of patients in this study is too small for reliable statistic analysis, there is an indication that DNA-ploidy may be a prognostic factor for the survival of patients with an adenocarcinoma in Barrett's oesophagus.

References

1. Menke-Pluymers MBE, Schoute NW, Mulder AH, Hop WCJ, van Blankenstein M, Tilanus HW. Outcome of surgical treatment of adenocarcinoma in Barrett's oesophagus. Gut 1992; 33: 1454-1458.
2. Skinner DB, Ferguson MK, Soriano A, Little AG, Staszak VM. Selection of operation for esophageal cancer based on staging. Ann Surg 1986; 204: 391-401.
3. Streitz JM, Ellis FH, Gibb SP, Balogh K, Watkins E. Adenocarcinoma in Barrett's esophagus. A clinico-pathological study of 63 cases. Ann Surg 1991; 213: 122-125.
4. Matsuura H, Sugimachi K, Ueo H, Kuwano H, Koga Y, Okamura T. Malignant potentiality of squamous cell carcinoma of the esophagus predictable by DNA analysis. Cancer 1986; 57: 1810-1814.
5. Ikebe M, Kitamura K, Baba K, Morita M, Tsutsui S, Sugimachi K. DNA ploidy as a prognostic factor in early esophageal carcinoma. Hepato-Gastroenterol 1993; 40: 232-235.
6. Albe X, Vassilakos P, Helfer-Guarnori K, et al. Independent prognostic value of ploidy in colorectal cancer. A prospective study using image cytometry. Cancer 1990; 66: 1168-1175.
7. Thorud E, Fossa SD, Vaage S, et al. Primary breast cancer. Flow cytometric DNA pattern in relation to clinical and histopathologic characteristics. Cancer 1986; 57: 808-811.
8. Klemi PJ, Joensuu H, Kiilholma P, Maenpaa J. Clinical significance of abnormal nuclear DNA content in serious ovarian tumours. Cancer 1988; 62: 2005-2010.
9. Aretxabala de X, Yonemura Y, Sugiyama K, et al. DNA ploidy pattern and tumour spread in gastric cancer. Br J Surg 1988; 75: 770-773.
10. Hedley DW, Friedlander ML, Taylor IW, Rugy CA, Musgrave EA. Method for analysis of cellular DNA content of paraffin embedded pathological material using flowcytometry. J Histochem 1983; 31; 1333-1335.
11. UICC (International Union Against Cancer). TNM classification of malignant tumours. Berlin: Springer-Verlag 1987.
12. Hiddeman W, Sluman J, Andreeff M, et al. Special report. Convention on nomenclature for DNA cytometry. Cytometry 1984; 5: 445-446.

13. Cox DR. Regression models and life tables. J Roy Statist Soc 1972; B 34: 187-220.
14. Hambraeus GM, Mercke CE, Willen R, et al. Prognostic factors influencing survival in combined radiotherapy and surgery of squamous cell carcinoma of the esophagus with special reference to a histopathological grading system. Cancer 1988; 62: 895-904.

CHAPTER 3

Risk factors for the development of an adenocarcinoma in Barrett's oesophagus

MBE Menke-Pluymers, WCJ Hop, J Dees, M van Blankenstein,
HW Tilanus

Published in:
Cancer 1993; 72: 1155-1158.

Summary

To evaluate the importance of the extension of columnar lined oesophagus, sex, age, smoking and drinking habits as risk factors for malignant degeneration, we performed a retrospective case-control study comparing patients with and without adenocarcinoma in Barrett's oesophagus.

The records of 96 patients (53 male and 43 female; mean age 61 years) with a benign columnar lined oesophagus and 62 patients (47 male and 15 female; mean age 62 years) with an adenocarcinoma in columnar lined oesophagus referred to the Rotterdam Oesophageal Tumour Study Group, diagnosed over the same period (1978-1985), were reviewed. A frequency distribution of the extension of columnar lined oesophagus in both groups was made. Statistical analysis was performed using multivariate methods.

The extension of columnar lined oesophagus was related significantly to carcinoma: a doubling of the length resulted in a 1.7 times increased risk. Smokers had a 2.3-fold increased risk as compared with nonsmokers. Male sex as a risk factor approached statistical significance ($p=0.006$). Adjusted for these risk factors, no relation between carcinoma and age or alcohol consumption was found.

In conclusion, the risk of development of an adenocarcinoma in Barrett's oesophagus increased with the extension of Barrett's epithelium. Smoking and possibly male sex were also risk factors. The identification of these risk factors may help in developing more efficient screening programs for patients with Barrett's oesophagus.

Introduction

Barrett's oesophagus is a condition wherein a variable length of squamous epithelium of the distal tubular oesophagus is replaced by columnar epithelium. After the first description of the condition in 1906 by Tileston (1), Barrett in 1950 described the columnar epithelium as occurring in a mediastinal stomach secondary to a congenital short oesophagus (2). Currently, the acquired origin of columnar epithelium in the distal tubular oesophagus secondary to gastro-oesophageal reflux is accepted most (3,4).

Adler was the first to stress the etiologic relationship between Barrett's oesophagus and oesophageal adenocarcinoma (5). The reported incidence of oesophageal cancer in patients with Barrett's oesophagus varies from one in 52 to one in 441 patient-years of follow-up (6-9). In comparison with the general population patients with Barrett's oesophagus run a 30 to 125 fold increased risk of development an oesophageal carcinoma (7-9). This is the reason why regular endoscopic screening has its advocates, although there is no agreement about the intensity and even the desirability of such a screening program (9-12). It is also an open question whether all patients with Barrett's oesophagus should be screened, or only a subgroup with special risk factors. A number of risk factors have been cited in the literature: race, age, sex, gastro-oesophageal reflux, intestinal metaplasia, dysplasia, extension of columnar epithelium in the oesophagus, and smoking and drinking habits (4,11-15). Harle et al. and Ransom et al. suggested a positive relationship between the extension of Barrett's epithelium and the risk of development of an adenocarcinoma in the oesophagus (15,16). According to this assumption, one would expect a higher frequency of longer segments of Barrett's epithelium in patients in whom adenocarcinoma develops compared with those without malignancy.

To evaluate the importance of the extension of columnar epithelium by analysis of the frequency distributions of the extension of Barrett's epithelium, sex, age, smoking and alcohol consumption as risk factors for development of adenocarcinoma in Barrett's oesophagus, we performed a retrospective case-control study, comparing patients with and without adenocarcinoma in Barrett's oesophagus.

Patients and methods

In this study, Barrett's columnar lined oesophagus was defined as the condition in which a segment of columnar epithelium is found at least 3 cm above the distal end of the tubular oesophagus as endoscopically defined. Cases of adenocarcinoma in Barrett's oesophagus were accepted only if at endoscopic examination the tumour was localized in the distal tubular oesophagus, if a segment of Barrett's columnar epithelium was found at or above the upper limit of the tumour, and if no tumour was visible in the gastric cardia at retroversion. Patients with carcinoma of the cardia were excluded from this study. In all cases, Barrett's oesophagus was proven histologically by biopsy specimens showing columnar epithelium. Adenocarcinoma also was proven histologically in all cases.

During the period 1978-1985, 96 consecutive cases of Barrett's oesophagus without carcinoma, meeting the above-mentioned criteria, were identified at endoscopy at our hospital. This group of patients with cancer-free Barrett's oesophagus consisted of 53 male and 43 female patients (male-female ratio 1.2:1), with a mean age of 61 years. Over the same period, 62 consecutive patients with an adenocarcinoma in Barrett's esophagus were referred (mostly from other hospitals) to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment. There were 47 male and 15 female patients (male-female ratio 3.1:1), with a mean age of 62 years.

For both groups, the extension of Barrett's oesophagus was measured from the lower end of the tubular oesophagus to the squamo-columnar junction identified at endoscopic examination. All measurements were performed by one of two experienced endoscopists. The demarcation between stomach and oesophagus was determined endoscopically by the diameter change of the lumen, termination of gastric folds, change in colour of the mucosa, and vascular pattern. The upper level was diagnosed by the epithelial border of Barrett's epithelium, which always was identified easily. In the group with carcinoma, all adenocarcinomas were localized in Barrett's epithelium. A frequency distribution was made for the extension of Barrett's oesophagus for both the group with benign Barrett's oesophagus and that with malignant disease. Data regarding smoking and alcohol consumption were recorded systematically as part of the medical history. Patients were designated as smokers if they smoked more than five cigarettes daily. Findings regarding alcohol consumption were counted as positive if there was a daily intake, and

intake of greater than four drinks per day was recorded.

In a case-control analysis, the risk factors were compared in both groups, representative for patients with Barrett's oesophagus with and without carcinoma, respectively, to obtain the relative risk. Statistical analysis included the Fisher exact test for comparing percentages and the Mann-Whitney test for comparison of continuous data. Various risk factors were evaluated simultaneously by logistic regression (17). A P value less than 0.05 (two-sided) was considered the limit of statistical significance.

Table 1. Characteristics regarding the sex-ratio, symptoms, smoking and alcohol consumption of the patients with a benign Barrett's oesophagaus (n=96), and the group with an adenocarcinoma in Barrett's oesophagus (n=62)*.

	Barrett's oesophagus	
	Benign	Malignant
	No.(%)	No.(%)
Male	53(55)	47(76)
Female	43(45)	15(24)
Presenting symptoms		
heartburn/regurgitation	23(24)	2(3)
dysphagia	26(27)	37(60)
pain	40(42)	15(24)
other	7(7)	8(13)
Smoking	28(31)	36(58)
Nonsmoking	63(69)	26(42)
Daily alcohol intake >4 drinks	2(2)	1(2)
Daily alcohol intake <4 drinks	40(44)	20(32)
No daily alcohol intake	48(53)	41(66)

* Missing data (only in the group with benign disease): smoking (n=5), alcohol consumption (n=6).

Results

There was no significant difference in the age distribution between both groups. In the group with benign Barrett's oesophagus, the median age was 65 years (range 14-96 years) and in the group with malignant disease 63 years (range 30-96 years). Table 1 summarizes the characteristics of both groups regarding sex ratio, symptoms, smoking and alcohol consumption. The percentage of smokers was significantly ($p<0.001$) higher in the group with malignant disease. The same applied to alcohol consumption ($p=0.02$). Also, the male to female ratio was significantly ($p=0.01$ greater in the group with malignant disease.

Figure 1. Frequency distributions of the length of Barrett's oesophagus for the groups with nonmalignant Barrett's oesophagus and malignant disease.

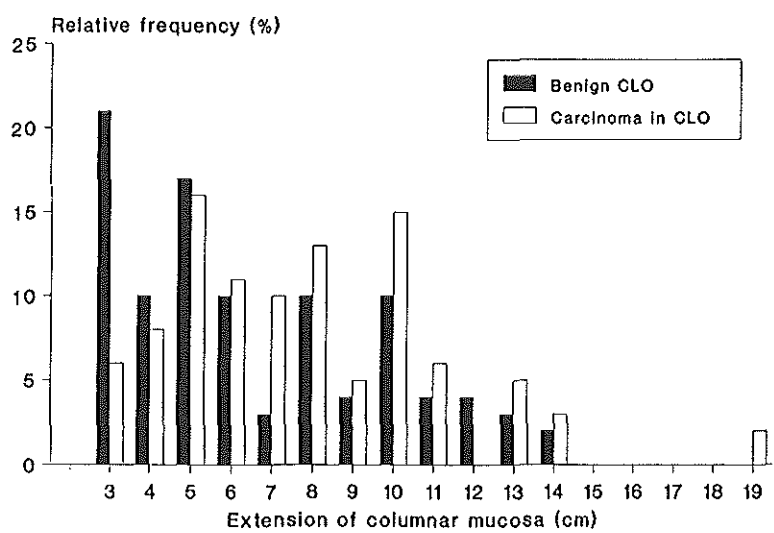


Figure 1 shows the frequency distributions of the extension of Barrett's oesophagus for the groups with nonmalignant Barrett's oesophagus and malignant disease. Forty percent of the patients with benign Barrett's oesophagus had a segment of columnar mucosa longer than 6 cm. In the group with an adenocarcinoma in Barrett's oesophagus,

60% of the patients had a length longer than 6 cm. By logistic regression, the odds ratio for each risk factor with respect to adenocarcinoma was obtained (Table 2). An odds ratio of 1.0 indicates no effect. An odds ratio greater than 1.0 indicates an increased risk of cancer in the group with this particular risk factor as compared with the group without the risk factor. A significantly increased risk of adenocarcinoma was associated with greater lengths of Barrrett's oesophagus: a doubling of any given length involved a 1.7 increase in risk ($p<0.05$). Smokers appeared to have a 2.3-fold increased risk. The 2.4-fold increased risk of development of an adenocarcinoma for male as compared with female patients, after allowing for the other factors, approached statistical significance ($p=0.06$). The group of patients reporting alcohol consumption did not have a significant increased risk in comparison with teetotalers. When the other risk factors were taken into account, no significant relation between adenocarcinoma and the age was found.

Table 2. Outcomes of multivariate analysis of various risk factors*.

Risk factor		Odds ratio	P-Value
Extension of BO		1.7#	<0.05
Sex:	female	1.0	
	male	2.4	0.06
Age		1.2#	
Alcohol:	no	1.0	
	yes	1.2	0.6
Smoking:	no	1.0	
	yes	2.3	<0.05

* Odds ratios and P values were calculated by logistic regression.

\# The odds ratio for the extension of Barrett's oesophagus (BO) applies to each doubling of the length. For the age factor, the risk increases with a factor 1.2 with a 10-year increase of age.

Discussion

Several studies have stressed the association between Barrett's oesophagus and adenocarcinoma (5-8). It is thought that longstanding gastro-oesophageal reflux damages the squamous epithelium of the lower tubular oesophagus, which is replaced by metaplastic columnar epithelium (18,19). This columnar epithelium may become dysplastic and eventually progress from mild to severe dysplasia into adenocarcinoma (9). In view of the high risk of adenocarcinoma, endoscopic surveillance for the detection of increasing dysplasia and malignant change has been recommended by some authors, although the screening program is expensive and time consuming (9,11). Others have questioned the value of a surveillance program for all patients with Barrett's oesophagus because the incidence in absolute terms is low and the influence on life expectancy is not well known (10,12). Therefore it seems important to detect subgroups of patients who are at risk for the development of adenocarcinoma and who will benefit from regular follow-up. Several risk factors that might enhance malignant degeneration, such as race, age, sex, gastro-oesophageal reflux, intestinal metaplasia, dysplasia, extension of columnar epithelium in the oesophagus, smoking and drinking habits, have been cited in the literature (4,11,13-15). It appears that Barrett's oesophagus occurs almost exclusively in white people (4,13).

The prevalence of oesophageal cancer in patients with Barrett's oesophagus varies from 7% to 33% (7,10,20). Because our hospital is a referral center for carcinoma of the oesophagus, the prevalence of adenocarcinoma in Barrett's oesophagus could not be determined in this study.

The average age at the time of diagnosis of Barrett's oesophagus ranges from 55 to 64 years (4,7,13-15,20-22). In most studies there is no significant difference in the mean age at onset of an adenocarcinoma in Barrett's oesophagus and the mean age at diagnosis of Barrett's oesophagus (4,7,14,21,22). This was also seen in our series.

There is a male predominance among patients with Barrett's oesophagus, with a male to female ratio varying from 2:1 to 4:1 (7,13-15,20). For patients with an adenocarcinoma in Barrett's oesophagus, the male to female ratio varies from 3:1 to 5.5:1 (4,13-15,22). We found a significant difference in the male to female ratio between the patients with benign Barrett's oesophagus and those with malignant disease with Barrett's

oesophagus. In the group with benign disease the male to female ratio was slightly greater than 1 (1.2:1), but in the group with malignant disease there was a definite male predominance, with a male to female ratio of 3.1:1.

It has been suggested that an extended columnar-lined oesophagus could increase the risk factor of development of an adenocarcinoma in Barrett's oesophagus (15,16). In a follow-up study of 155 patients with Barrett's oesophagus performed at our institution, adenocarcinoma was found to have developed in four patients with longest segments of Barrett's mucosa (10). However, in the current series, 40% of the patients with an adenocarcinoma had a segment of columnar lined oesophagus of 6 cm or less, showing that adenocarcinoma also occurs in shorter segments of Barrett's oesophagus. Our data indicate that for each doubling of the length the risk of development of an adenocarcinoma increases by a factor of 1.7.

Smoking and alcohol consumption usually are considered to be risk factors for the development of carcinoma of the oesophagus (12,13,23). Smoking and alcohol consumption were reported by a higher percentage of patients with an adenocarcinoma as compared with patients with benign Barrett's oesophagus. However, after correction for the other risk factors, smokers had a significantly higher risk than non-smokers, whereas there was no significantly increased risk from alcohol consumption. This is in accordance with several studies in the literature, which identified a correlation between smoking and adenocarcinomas in Barrett's oesophagus, but not between alcohol consumption and carcinoma (13,23).

In summary, this case-control study shows that the risk of development of an adenocarcinoma in Barrett's oesophagus increases with the extension of Barrett's epithelium by a factor of 1.7 for each doubling of the length of Barrett's epithelium. Smoking and possibly male sex were also risk factors, whereas after correction for the extension of Barrett's oesophagus, age, and smoking there was no significantly increased risk from alcohol consumption. The identification of risk factors for the development of adenocarcinoma in Barrett's oesophagus may help in creating more efficient screening programs.

References

1. Tileston W. Peptic ulcer of the esophagus. Am J Med Sci 1906; 132:240-265.
2. Barrett NR. Chronic peptic ulcer of the oesophagus and oesophagitis. Br J Surg 1950; 38:175-182.
3. Allison PR, Johnstone AS. The oesophagus lined with gastric mucous membrane. Thorax 1953; 8:87-101.
4. Sarr MG, Hamilton SR, Marrone GC, Cameron JL. Barrett's esophagus: it's prevalence and association with adenocarcinoma in patients with symptoms of gastro-oesophageal reflux. Am J Surg 1985; 149:187-193.
5. Adler RH. The lower esophagus lined by columnar epithelium. It's association with hiatal hernia, ulcer, stricture and tumor. J Thorac Cardiovasc surg 1953; 45:13-34.
6. Sprung DJ, Ellis FH Jr., Gibb SP. Incidence of adenocarcinoma in Barrett's esophagus. Am J Gastroenterology 1984; 79: 817.
7. Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. N Engl J med 1985; 313:857-858.
8. Spechler SJ, Goyal RK. Barrett's esophagus. N Engl J Med 1986; 315;362-371.
9. Hameeteman W, Tytgat GNJ, Houthoff HJ, Van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989; 96:1249-1256.
10. Van der Veen AH, Dees J, Blankensteyn JD, Blankenstein M van. Adenocarcinoma in Barrett's oesophagus: an overrrated risk. Gut 1989; 30:14-18.
11. Robertson CS, Mayberry JF, Nicholson DA, James PD, Atkinson M. Value of endoscopic surveillance in the detection of neoplastic change in Barrett's esophagus. Br J Surg 1988;75:760-763.
12. Spechler SJ, Robins AH, Rubins HB, et al. Adenocarcinoma and Barrett's esophagus: an overrated risk? Gastroenterology 1984; 87:927-933.
13. Skinner DB, Walther BC, Ridell RH, Schmidt H, Iascone C, DeMeester T. Barrett's esophagus. Comparison of benign and malignant cases. Ann Surg 1983; 198:554-566.

14. Starnes VA, Adkins RB, Ballinger JF, Sawyers JL. Barrett's esophagus. A surgical entity. Arch Surg 1984; 119:563-567.

15. Harle IA, Finley RJ, Belsheim M, et al. Management of adenocarcinoma in columnar-lined oesophagus. Am Thorac Surg 1985; 40;330-336.

16. Ransom JM, Patel GR, Cliff SA, Wamble NE, Read RC. Extended and limited types of Barrett's esophagus in the adult. Am Thorac Surg 1982; 33:19-27.

17. Cox DR. The analysis of binary data, 1970, London, Methuan.

18. Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TPJ. Experimental columnar metaplasia in the canine oesophagus. Br J Surg 1988; 75:113-115.

19. Williamson WA, Ellis FH, Gibb SP, Shahian DM, Aretz HT. Effect of antireflux operation on Barrett's mucosa. Ann Thorac Surg 1990; 49:537-542.

20. Polepalle SC, McCallum RW. Barrett's esophagus. Current assessment and future perspectives. Gastroenterology Clinics of North America 1990; 19(3):733-744.

21. Gillen P, Keeling P, Hennessy TPJ. Barrett's oesophagus: risk faactors for malignancy (Abstract). Gut 1987; 28: A1379.

22. Bremner CG. The management of columnar-lined oesophagus. In: Surgery of the oesophagus, Jamieson GG (ed.), Churchill Livingstone, 1988.

23. Gray MR, Kingsnorth AN. Smoking and cancer risk in Barrett's columnar lined oesophagus (Abstract). Gut 1990, A T56.

CHAPTER 4

Dysplasia and aneuploidy as markers of malignant degeneration in Barrett's oesophagus

MBE Menke-Pluymers, AH Mulder, WCJ Hop, M van Blankenstein,
HW Tilanus

Published in:
Gut 1994; 35: 1348-1351.

Summary

The role of dysplasia and aneuploidy as markers in columnar epithelium for malignant degeneration in Barrett's oesophagus was compared in a case control study comprising 38 patients with a benign Barrett's oesophagus and 50 patients with Barrett's oesophagus associated with adenocarcinoma. Tissue specimens of columnar epithelium were reviewed for the presence of specialized columnar epithelium and the grade of dysplasia. Ploidy was determined using the method for formalin fixed paraffin embedded tissue described by Hedley.

There was no significant difference in the frequency of specialized columnar epithelium between both groups. Dysplasia was more often found in columnar epithelium associated with adenocarcinoma as compared to benign Barrett's oesophagus ($p<0.001$). Multivariate analysis using logistic regression revealed an increased risk of malignancy in Barrett's oesophagus in case of dysplasia (odds ratio 9.4, $p=0.003$ for mild dysplasia and 33.1, $p<0.001$ for moderate or severe dysplasia). DNA-ploidy was not statistically significant correlated with dysplasia. Aneuploidy or increased G2/tetraploidy proved to be an independent risk factor for younger patients (age $<$ 65 years: odds ratio 44.7, $p=0.003$).

In conclusion, dysplasia and aneuploidy or increased G2/tetraploidy in columnar epithelium are independent risk factors for malignant degeneration. Patients with these risk factors should be offered a more intensive screening programme.

Introduction

Barrett's oesophagus represents a metaplastic transformation of the normal squamous cell epithelium of the lower tubular oesophagus into columnar epithelium (1,2). The metaplastic columnar epithelium has the potential to undergo dysplastic changes resulting in adenocarcinoma (3). There is an increased risk of adenocarcinoma developing in Barrett's oesophagus (4). This risk is estimated to be 30-125 fold higher than in the general population (3,5). The value of endoscopic surveillance has not been fully established and it has been suggested it should be concentrated on a subgroup of high risk patients (6). It would therefore be valuable to discover markers identifying this subset of patients. Histological changes (both specialized columnar epithelium and dysplasia) and flow cytometric abnormalities have been suggested as putative markers of increased risk of malignancy in Barrett's oesophagus (3,7-10). However, the only screening marker in clinical use at the moment is the presence of dysplasia. One group concluded that flow cytometry is capable of detecting alterations in DNA-content which are present in high frequency in Barrett's oesophagus with dysplastic changes and carcinoma (8,9). Others, however, have found discordance between flow cytometric abnormalities and dysplasia in Barrett's oesophagus (11). It remains unclear whether the presence of both aneuploidy and dysplasia implies a greater cancer risk than each factor separately or that they merely represent confirmatory markers.

To evaluate the association of dysplasia and aneuploidy with malignant change in Barrett's oesophagus, we performed a case control study comparing the occurrence of dysplasia and aneuploidy in columnar epithelium in patients with adenocarcinoma in Barrett's oesophagus to an age and sex matched group of patients with Barrett's oesophagus, recruted during the same period, in whom no carcinoma developed during a follow-up period of at least 5 years.

Patients and methods

Definitions.

Barrett's oesophagus was defined as the condition in which a segment of columnar epithelium was found of at least three centimeters above the distal end of the tubular

oesophagus as endoscopically defined. In all cases columnar epithelium was proven histologically by endoscopically directed biopsies demonstrating columnar epithelium. Cases of adenocarcinoma in Barrett's oesophagus were only accepted if a segment of Barrett's columnar epithelium was found at or above the upper limit of the tumour and if the bulk of the tumour was located in the tubular oesophagus. All cases of adenocarcinoma were confirmed by histologic examination of biopsies and resection specimens.

Patients.

During the period 1978-1988, 112 consecutive patients with an adenocarcinoma in Barrett's oesophagus were referred to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment. Tissue specimens of columnar epithelium from 50 of these patients (biopsies in 36 cases and resection specimens in 14) were available for flowcytometric analysis. They comprised 36 men and 14 women with a mean age of 62.2 years (range 39-81), and constituted our study group with malignant change in Barrett's oesophagus. Thirty-eight patients underwent oesophagectomy, of whom 24 had received preoperative radiation therapy. In 6 patients the tumour proved unresectable at laparotomy and 6 patients were not considered for resection because of distant metastases. Resection specimens from the 24 patients who had received preoperative radiotherapy were discarded, but in these cases biopsy specimens of columnar epithelium obtained before radiotherapy were analyzed. Thirty-eight patients with a columnar lined oesophagus and with a similar age and sex distribution, who entered the surveillance programme between 1980 and 1986, constituted the control group. These patients were 28 men and 10 women with a mean age of 62.6 years (range 30-88), none of whom developed an oesophageal carcinoma during a follow-up period of 5-11 years. Endoscopic biopsies obtained at the first endoscopy were used for histological examination and flow cytometric analysis. Histological examination and flow cytometry were always performed on the same specimen.

Endoscopy and Biopsy.

All patients underwent endoscopy using an Olympus GIFQ 10 endoscope. Biopsy specimens were taken at one centimeter intervals throughout the columnar lined tubular oesophagus up to the squamocolumnar junction and directed biopsies were taken of any endoscopic abnormality. All endoscopically directed biopsies were embedded in one paraffin block, which was used for analysis.

Histology.

All tissue specimens of columnar epithelium obtained by endoscopically directed biopsies (usually 3-8 biopsies in one paraffin specimen) or resection (several cross-sections through the oesophagus) were reviewed by two of us (AH M. and MBE MP.). Specimens were designated as no dysplasia, mild, moderate or severe dysplasia according to criteria for epithelial dysplasia in the stomach described by Morson et al. (12). Since severity of dysplasia may be patchy and the paraffin blocks contained several biopsies, the most severe type of dysplasia was scored. The type of columnar epithelium was determined using criteria described by Paull et al. (13).

Specimens.

All specimens were either endoscopically directed biopsies or resection specimens of columnar epithelium fixed in formalin and embedded in paraffin. These specimens were prepared for flowcytometry according to the method described by Hedley (14). The nuclear suspension was filtered through a 40 micrometer mesh filter and stained with ethidium bromide (BDH Chemicals Ltd. Poole England). Cellular DNA-content of at least 10.000 cells was measured on a fluorescence activated cell sorter (FACS II Becton Dickinson, Sunnyville, California, USA). The Argon laser was tuned at 488 nm and a red fluorescence was measured using a cut off filter (Pomfrett Stamford, Cann.). The data were fed into a Data General computer.

Flowcytometry analysis.

Aneuploidy was determined both by visual inspection and confirmation of an aneuploid population by calculating the deoxyribonucleic acid index as defined by the Convention of Nomenclature for deoxyribonucleic acid cytometry (15). A diploid histogram contained a G0/G1 peak (DNA-index 1.00) and a G2/tetraploid peak above DNA-index 1.90 and below DNA-index 2.10, with a G2/tetraploid fraction up to 10%. G2/tetraploid fractions above 10% were considered abnormal. Specimens were excluded from analysis if the coefficient of variation exceeded 9% or the cells numbered less than 10000 for analysis.

Statistical analysis.

Statistical analysis included Mann-Whitney's test for the comparison of continuous data and Fisher's exact test for the comparison of percentages. Dysplasia and aneuploidy as risk factors for malignant change were evaluated simultaneously by a multivariate analysis using logistic regression (16). $P=0.05$ (two-sided) was considered the limit of statistical

significance.

Results

There was no significant difference ($p=0.35$) in the frequency in which specialized columnar epithelium was found in both groups: 29 cases (76%) in the benign group and 33 cases (66%) in the malignant group. Table 1 shows the grades of dysplasia for both groups. The frequency and grade of dysplasia found in Barrett's epithelium associated with adenocarcinoma were higher than that in benign Barrett's epithelium ($p<0.001$).

Table 1. Grade of dysplasia of columnar epithelium of patients with benign Barrett's oesophagus and patients with Barrett's oesophagus associated with adenocarcinoma.

	Benign Group N (%)	Malignant Group N (%)
No dysplasia	25 (66)	9 (18)
Mild dysplasia	11 (29)	19 (38)
Moderate dysplasia	2 (5)	14 (28)
Severe dysplasia	-	8 (16)

Table 2 summarizes the results of flow cytometry. Ploidy did not significantly correlate with the grade of dysplasia in columnar epithelium in either group. Aneuploidy or increased G2/tetraploidy of columnar epithelium occurred significantly more often ($p<0.001$) in the malignant group (62%) as compared to the benign group (21%).

Using multivariate analysis, dysplasia and aneuploidy or increased G2/tetraploidy were simultaneously evaluated as risk factors with respect to adenocarcinoma (Table 3). Odds ratios of 1 indicate no effect. An odds ratio above 1 indicates an increased risk of cancer in the group with this particular risk factor as compared to the group without the risk factor. A significantly increased risk of adenocarcinoma was associated with dysplasia, involving a 9-fold increased risk for mild dysplasia ($p=0.003$) and a 33-fold increased risk for moderate

or severe dysplasia ($p<0.001$). Aneuploidy or increased G2/tetraploidy appeared to be a significant ($p=0.003$) independent risk factor for malignancy in Barrett's oesophagus for younger patients (age < 65 years), whereas the risk increase was not significant for older patients (age > 65 years). The effects of the various risk factors were not affected by gender.

Table 2. Results of flowcytometry of columnar lined epithelium of patients with benign Barrett's oesophagus and of patients with Barrett's oesophagus associated with adenocarcinoma according to grade of dysplasia.

	Diploid N (%)	Aneuploid Increased G2/tetraploid N (%)
Benign Group		
no dysplasia	21 (84)	4 (16)
mild dysplasia	9 (82)	2 (18)
moderate/severe dysplasia	-	2 (100)
Total	30 (79)	8 (21)
Malignant Group		
no dysplasia	1 (11)	8 (89)
mild dysplasia	11 (58)	8 (42)
moderate/severe dysplasia	7 (32)	15 (68)
Total	19 (38)	31 (62)

Discussion

A number of reports have stressed the association between Barrett's oesophagus and adenocarcinoma (3-6). The metaplastic columnar epithelium may progress from mild to severe dysplasia into adenocarcinoma (3). In view of the 30-125 -fold increased risk of adenocarcinoma regular endoscopic and histologic surveillance have been recommended, although there is no agreement about the intensity of such a screening programme (3,5). Because of the low incidence of carcinoma it should be important to identify the subset of

Table 3. Outcomes of multivariate analysis of the risk factors dysplasia and aneuploidy.

Risk factor	Odds ratio	P-value
No dysplasia[a]	1	
Mild dysplasia	9.4	0.003
Moderate/severe dysplasia	33.1	<0.001
Diploid[a]	1	
Aneuploid/increased G2 tetraploid		
age < 65 years	44.7[b]	0.003
age > 65 years	2.4	0.28

(a) Reference category

(b) Significantly (p=0.03) difference between ages above or below 65 years.

patients with Barrett's oesophagus at high risk for the development of adenocarcinoma and therefore to concentrate surveillance on high risk patients. Most endoscopic surveillance protocols are based on histologic evaluation of biopsy specimens for dysplasia (17). However, the morphologic diagnosis of dysplasia, especially that of lesser degrees is difficult and marred by considerable intra- and interobserver variation. Therefore more objective indicators would be useful.

In most patients with an adenocarcinoma in Barrett's oesophagus dysplasia is found in the surrounding columnar epithelium (8,10,20). Schmidt et al. found high grade dysplasia in 48% and low grade dysplasia in 30% of the patients with an adenocarcinoma in Barrett's oesophagus compared with only low grade dysplasia in 5% of the patients without carcinoma (10). This agrees with our study, in which we found moderate or severe dysplasia in 44% of the patients in the malignant group but only moderate dysplasia in 5% of the patients in the benign group.

The relationship between flowcytometry and dysplasia is controversial. Reid et al. demonstrated genomic instability (aneuploidy) or abnormalities of mucosal proliferation by flow cytometry in specimens of patients with Barrett's oesophagus with dysplasia and

adenocarcinoma (7). They showed that flow cytometric abnormalities were correlated with the histologic diagnosis of dysplasia and carcinoma. In contrast, Fennerty et al. found that histologic dysplasia and aneuploidy were often discordant (11). In our study ploidy did not significantly correlate with the grade of dysplasia in columnar epithelium in either group, although specimens with moderate or severe dysplasia showed a trend towards aneuploidy or increased G2/tetraploidy. Recently Reid et al. demonstrated that most of the patients whose tissues showed aneuploidy or increased G2/tetraploid fractions progressed to high grade dysplasia or carcinoma regardless of their histological dysplastic abnormalities (9). On the basis of these data it is still unclear whether flow cytometry and histology are complimentary risk factors, or merely serve as confirmatory markers.

Our study was designed to evaluate the role of dysplasia and aneuploidy as independent risk factors and to determine the extent of this risk. Our data indicate that the relative risk increases 9-fold for mild dysplasia and 33-fold for moderate or severe dysplasia. For younger patients (age $<$ 65 years) aneuploidy or increased G2/tetraploidy appeared to be a significant risk factor for malignant degeneration with a 45-fold increased relative risk. In the benign group the 8 cases with aneuploidy or increased G2/tetraploidy had a significant higher ($p=0.04$) mean age than those with diploidy (mean ages respectively 61 and 69 years). In the malignant group there was no age difference, with means both equalling 62 years. There was no relation between age and dysplasia score in either group. For columnar lined epithelium no relation between ploidy and age has been described in contrast to some studies on flowcytometric analysis of nuclear DNA content in ovarian tumours and breast adenocarcinoma which revealed an association between aneuploidy and advanced age (18,19). Specialized columnar epithelium was found with equal frequency in benign Barrett's mucosa and Barrett's mucosa associated with adenocarcinoma.

In conclusion, an increased risk for malignant degeneration was found where dysplasia was present in columnar epithelium, especially moderate and severe dysplasia. Ploidy was not significantly correlated with dysplasia. Aneuploidy or increased G2/tetraploidy, however, proved to be an independent risk factor in patients under 65 years of age. On the basis of our finding that both dysplasia and aneuploidy or increased G2/tetraploidy are independent risk factors for malignant degeneration in Barrett's oesophagus, patients with either or both these risk factors should be offered at least annual or half yearly screening, provided they are able to undergo surgical intervention. As severe dysplasia is often associated with invasive

carcinoma, these patients should be offered oesophageal resection or at least frequent (three monthly) endoscopies with extensive biopsies (17,20). Patients without these factors may be screened at greater intervals guided by other risk factors (21).

References

1. Barrett NR. The lower oesophgaus lined by columnar epithelium. Surgery 1957;41:881-894.
2. Allison PR, Johnstone AS. The oesophagus lined with gastric mucous membrane. Thorax 1953;8:87-101.
3. Hameeteman W, Tytgat GNJ, Houthoff HJ, Van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989;96:1249-1256.
4. Dees J, van Blankenstein M, Frenkel M. Adenocarcinoma in Barrett's oesophagus: a report of 16 cases. Gut 1978;19: A 976.
5. Cameron AJ, Ott BJ, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus.
   N Engl J Med 1985;313:857-858.
6. Van der Veen AH, Dees J, Blankensteyn JD, van Blankenstein M. Adenocarcinoma in Barrett's oesophagus: an overrated risk. Gut 1989;30:14-18.
7. Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. Barrett's esophagus. Correlation between flow cytometry and histology in the detection of patients at risk for adenocarcinoma. Gastroenterology 1987;93:1-11.
8. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett's esophagus is associated with genomic instability. Lab Invest 1988;60(1):65-71.
9. Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC, Rabinovitch PS. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenetrology 1992;102:1212-1219.
10. Schmidt HG, Riddell RH, Walther B, Skinner DB, Rieman JF. Dysplasia in Barrett's esophagus. J Cancer Res Clin Oncol 1985;110:145-152.
11. Fennerty MB, Sampliner RE, Way D, Riddell R, Steinbronn K, Garewal HS. Discordance between flow cytometric abnormalities and dysplasia in Barrett's esophagus. Gastroenterology 1989;97:815-820.

12. Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol 1980;33:711-721.
13. Paull A, Trier JS, Dalton MD, Camp RC, Loeb P, Goyal RK. The histopathologic spectrum of Barrett's esophagus. N Engl J Med 1976;295(9):476-480.
14. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method for analysis of cellular DNA content of paraffin embedded pathological material using flow cytometry. J Histochem Cytochem 1983;31(11):1333-1335.
15. Hiddeman W, Sluman J, Andreeff M, et al. Special report. Convention on nomenclature for DNA-cytometry. Cytometry 1984;5:445-446.
16. Cox DR. The analysis of binary data, p 87-91. London: Methuan 1970.
17. Reid BJ, Weinstein WM, Lewin KJ, Haggitt RC, VanDeventer G, DenBesten L, Rubin C. Endoscopic biopsy can detect high grade dysplasia or early adenocarcinoma in Barrett's esophagus without grossly recognizable neoplastic lesions. Gastroenterology 1988;94:81-90.
18. Klemi PJ, Joensuu H, Kiilholma P, Maenpaa J. Clinical significance of abnormal nuclear DNA content in serious ovarian tumors. Cancer 1988;62:2005-2010.
19. Taylor IW, Musgrove EA, Friedlander ML, Foo MS, Badley DW. The influence of age on the DNA ploidy levels of breast tumors. Eur J Cancer Clin Oncol 1983;19:623-628.
20. Pera M, Trastek VF, Carpenter HA, Allen MS, Deschamps C, Pairolero PC. Barrett's esophagus with high grade dysplasia: an indication for esophagectomy ? Ann Thorac Surg 1992;54:199-204.
21. Menke-Pluymers MBE, Hop WCJ, Dees J, van Blankenstein M, Tilanus HW. Risk factors for the development of an adenocarcinoma in columnar-lined (Barrett's) esophagus. Cancer 1993;72:1155-1158.

CHAPTER 5

Cytogenetic analysis of Barrett's mucosa and adenocarcinoma of the distal oesophagus and cardia.

MBE Menke-Pluymers, E van Drunen, KJ Vissers, AH Mulder,
HW Tilanus, AMMJ Hagemeijer-Hausman.

Cancer Genetics and Cytogenetics, in press.

Summary

We performed flowcytometry and cytogenetic analysis of 37 adenocarcinomas of the distal oesophagus and cardia of which 22 arose in Barrett's mucosa. Two of eight analyzed specimens of Barrett's mucosa had clonal chromosomal abnormalities. In 19 cases clonal chromosomal abnormalities were found in tumour tissue. The complex pattern of cytogenetic changes did not differ among the adenocarcinomas arisen in Barrett's oesophagus, and those in the distal oesophagus without Barrett's mucosa or cardia. Abnormal karyotypes with multiple and complex rearrangements were seen in 11 cases and with single or a few numerical changes in 8. Losses of chromosomes 4, 18, 21 and Y were the most frequent numerical changes. Loss of the Y-chromosome was observed in 8 of 26 tumours of males (31%). Gains of chromosomes 14 and 20 were also frequent numerical changes. Structural abnormalities were observed in 13 of the abnormal karyotypes (68%). The chromosome arms most frequently rearranged were 1p, 3q, 11p and 22p. The chromosome arm most frequently contributing to losses was 1p with the shortest region of overlap being 1p22-33. The chromosome arms most often involved in gains were 11p and 22p, and i(3q) was the isochromosome that was most frequently identified.

Introduction

There has been a striking increase in the incidence of adenocarcinoma of the oesophagus and the oesophagogastric junction in North America and Western Europe in recent years (1,2). Most oesophageal and gastro-oesophageal adenocarcinomas appear to arise from Barrett's oesophagus (3). As a consequence of persistent damage, the squamous epithelial lining of the oesophagus is replaced by columnar epithelium. The incidence of adenocarcinoma to develop in a patient with Barrett's oesophagus varies from 1 in 56 to 1 in 441 patientyears of follow-up (4). Although the risk of developing an adenocarcinoma appears to be small, it is at least 30-125 times that expected for a similar population without Barrett's oesophagus. The survival of patients with an oesophageal adenocarcinoma is poor, even after oesophagectomy (5,6). Although endoscopic surveillance and biopsy are performed to achieve an early diagnosis of malignant degeneration, its value has not been fully established and it has been suggested to concentrate on a subgroup of high risk patients (7). A better understanding of the underlying mechanisms of carcinogenesis in Barrett's oesophagus and the discovery of markers identifying the subset of patients at high risk would be of great importance.

Barrett's adenocarcinoma occurs as a result of progression of severe dysplastic changes in the abnormal mucosal lining of the oesophagus, involving a series of genetic alterations in the cells that comprise the Barrett's epithelium (8). The correlation between dysplastic changes and aneuploidy has been demonstrated by flowcytometric techniques (8,9). A few studies demonstrated clonal chromosome abnormalities in Barrett's epithelium and adenocarcinoma (10-13). The karyotypes were often complex, but loss of the Y-chromosome was a frequent finding. Genetic changes affecting 3q and the 11p13-15 region have been described in oesophageal adenocarcinomas as well as in other tumours (11,12). However, since the cytogenetic study of solid tumours and premalignant tissues is difficult, there are no large series reported.

We report cytogenetic studies of 37 adenocarcinomas of the distal oesophagus and cardia of which the majority (59%) arose in Barrett's mucosa. In these cases (n=22) cytogenetic analysis was also performed on Barrett's epithelium.

Materials and methods

Patients.

Patients with an oesophageal carcinoma were referred to the Rotterdam Oesophageal Tumour Study Group for evaluation and treatment. Three groups were investigated: 1) patients with an adenocarcinoma in Barrett's oesophagus, 2) patients with an adenocarcinoma in the distal oesophagus without Barrett's mucosa and 3) patients with an adenocarcinoma in the cardia. All patients underwent an oesophagectomy without preoperative radiotherapy or chemotherapy and were included in the study during the period November 1989 to August 1992. Tissue specimens of 37 patients (26 male and 11 female; mean age of 62 years) were analyzed by histopathological, flowcytometric and cytogenetic methods.

Pathology.

Based on histopathologic findings and site of the tumour the 37 adenocarcinomas of the patients were divided in three subgroups:

1) Adenocarcinoma in Barrett's oesophagus, if the bulk of the tumour was located in the distal oesophagus and columnar epithelium was found proximal to the tumour at microscopical examination (N=22).

2) Adenocarcinoma in the distal oesophagus, if the bulk of the tumour was located in the distal oesophagus and no columnar epithelium could be demonstrated proximal to the tumour by microscopical examination (N=10).

3) Adenocarcinoma of the cardia, if the bulk of the tumour was located in the cardia with tumourgrowth into the distal oesophagus (N=5).

From all 37 resection specimens fresh tissue samples of the oesophageal mucosa and the tumour were taken out and divided in 3 parts. One part of every tissue sample was used for histopathological analysis, one for cytogenetic analysis and a third part was stored in liquid nitrogen (-70°C) for flowcytometry. In a few cases tissue samples were taken from lymph node metastases.

If columnar epithelium was found, specimens were designated as no dysplasia, mild-, moderate-, and severe dysplasia according to criteria for epithelial dysplasia in the stomach described by Morson et al (14). In all tumour specimens adenocarcinoma was found and the differentiation grade of the tumour was determined.

Flowcytometry (FCM).

Fresh tissue samples for FCM were immediately frozen and stored in liquid nitrogen (-70°). These specimens were prepared for FCM according to the method described by Vindelov et al. (15). The nuclear suspension was filtered through a 40 micrometer mesh filter and stained with propidium iodide (Sigma Chemical Co., St. Louis, MO). Cellular DNA content of at least 10,000 cells was measured on FACS scan (Beckton Dickinson, Mountainview, California). The data were fed into a Data General computer. Aneuploidy was determined both by visual inspection of the histograms and confirmation of an aneuploid population by calculating the deoxyribonucleic acid index as defined by the convention of nomenclature for deoxyribonucleic acid (16). A diploid histogram contained a G0/G1 peak and a G2/tetraploid peak above DNA index 1.90 and below DNA index 2.10, with a G2/tetraploid fraction up to 10%. G2/tetraploid fractions above 10% were considered abnormal. An aneuploid population of cells was defined as a G0/G1 population of cells that produced a discrete peak, separate from the diploid population, constituting 5% or more of the cells in the biopsy specimens. The coefficient of variation was 6% or less in all cases.

Cytogenetics.

Fresh tissue samples of oesophageal mucosa and adenocarcinoma obtained from the resection specimens were minced with sharp scissors into pieces approximately 1 mm or less in diameter. The pieces were incubated in DMEM/Ham's F12 culture medium containing 5 ug/ml Amphotericin B, 200 IU/ ml penicillin and 0.2 mg/ml streptomycin for one hour. The suspension was washed in medium, then medium containing collagenase II (200 IU/ml) was added for several hours in order to obtain enzymatical disaggregation of cells. The suspension was washed two times in medium and dispersed into 50 ml cell culture T-flasks with 10 ml DMEM/ Ham's F12 culture medium with 10% fetal calf serum, 0.3 mg/ml glutamin, 100 IU/ml penicillin and 0.1 mg/ml streptomycin. The cultures were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide in air. The specimens were harvested for cytogenetic analysis after 2 or 3 days of growth in vitro (short term culture). To synchronize the cells in the cellcyclus the cultures were treated with methotrexate (1 ug/ml) overnight and tymidine (10^{-5}M) 6 hours before harvesting (17). Metaphase cells were arrested by exposure to colcemid (0.1 ug/ml) for 1 hour. The mitotic cells were loosened by shaking the culture flasks, washed with medium, swelled in KCl 0.075 M at 37°C for 8 minutes and fixed according to standard procedures using methanol:acetic acid (3:1). The remaining monolayer

was flooded with KCl-EGTA for 20 minutes and scraped out with a rubber policeman, washed with KCl 0.075 M and processed as above. The chromosomes were identified using RFA and QFQ banding. Karyotype designations were in accordance with the ISCN (1991) (18). As a control, preoperative blood samples of the patients were obtained and cytogenetic analysis of the leucocytes was performed.

The most frequent problems we encountered in processing tissue cultures for cytogenetic analysis were lack of outgrowth, contamination of the tissue cultures most often by yeast or fibroblast overgrowth. Incubating the minced tissue pieces in medium containing 5 ug/ml Amphotericin B directly after preparation of the resection specimens minimalized the contamination by yeast. We used only short term cultures (2-3 days) to prevent karyotypic evolution in vitro. The direct method, i.e. processing the tissue samples for cytogenetic analysis without tissue culture, yielded an insufficient number of metaphase cells. The quality of metaphases was irregular and not all cells could always be fully karyotyped.

Results

Clinical characteristics.

Table 1 shows the clinical and histopathological characteristics of 22 patients with an adenocarcinoma in Barrett's oesophagus. The histological diagnosis of the mucosal specimens on which cytogenetic and flowcytometric analysis were performed are listed according to the mucosal type, i.e. Barrett's epithelium or squamous epithelium. In one patient (no. 19) tumour tissue for cytogenetic analysis was obtained from a lymph node metastasis, while the tissue culture of the primary tumour was not successful.

Barrett's mucosa.

The results of flowcytometric (12 cases) and cytogenetic analysis (8 cases) of Barrett's mucosa are listed in table 2. FCM showed a diploid population in 8 cases, a tetraploid population in one and aneuploidy in 3 cases. Of these three aneuploid populations no cytogenetic analysis could be performed in patient no. 9, only 3 mitoses were analyzed and not clonally abnormal in patient no. 24, while in patient no. 25 clonal abnormalities were found. In this patient the DNA-index of Barrett's mucosa was 1.61, correlating with the modal chromosome number (near-triploid) found at cytogenetic analysis. The same clonal

karyotypic aberrations were also seen in the adenocarcinoma of the same patient. One of the diploid samples of Barrett's mucosa showed a hypodiploid karyotype with loss of the Y-chromosome. Loss of Y was also seen in the adenocarcinoma of this patient. The number of metaphases was insufficient in 4 cases (less than 5 mitoses), and in none of them an abnormal clone was found, despite the fact that FCM showed aneuploidy in one case. In three patients the mucosal tissue samples that were analyzed cytogenetically contained only squamous epithelium. In these cases Barrett's mucosa seen histologically in their resection specimens was not present in the tissue samples for cytogenetic analysis. These samples all showed normal karyotypes (3,8 and 4 metaphases were analyzed respectively). In 11 patients out of 22, tissue cultures of the mucosa were not successful which prevented cytogenetic analysis.

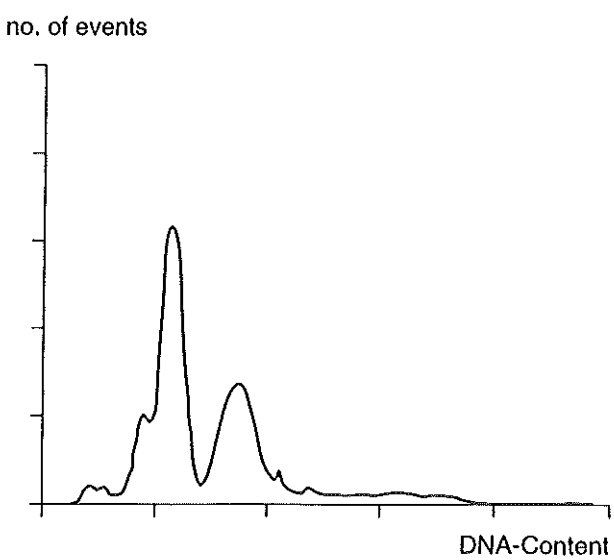
Adenocarcinoma.

In table 3 the data of flowcytometric and cytogenetic analysis of 22 adenocarcinomas in Barrett's oesophagus are summarized. FCM showed only diploid populations in 2, a tetraploid population in one and aneuploidy in 14 cases. In the latter the DNA indices varied between 1.42 and 1.72 in 10 cases. Four of these 10 tumours showed also near-triploid karyotypes (no.19,25,31 and 36). Patient 13 exhibited two distinct clonal populations, one hypodiploid and the second one near-triploid, both showing the same markers. Of the 12 cytogenetic abnormal clones identified, 5 were hypodiploid and two of these corresponded to near-triploid FCM indices, a situation similar to that of patient 13. There were no mitoses found in one case, and an inadequate number of metaphases in 4 cases, in which no abnormal clones were found, although FCM showed aneuploidy in two. In 9 tumours between 5 and 10 metaphases were karyotyped and an abnormal clone was found in 4 cases. In 4 tumours aneuploidy or tetraploidy was found by FCM, but this could not be confirmed cytogenetically.

Table 4 shows the clinical and histopathological characteristics of the patients with an adenocarcinoma in the distal oesophagus without Barrett's mucosa (n=10) and cardia (n=5). In two patients the tumour appeared to be irresectable at laparotomy and tumour tissue was obtained from lymph node metastases (no.7 and 8). All oesophageal mucosa specimens were of the squamous cell type. Cytogenetic analysis of squamous epithelium was performed in 6 cases (1-5 metaphases were analyzed in 4 cases and 10 metaphases in 2 cases), and at random numerical changes were found in 4 of them. In the other 9 patients tissue cultures of the squamous epithelium were not successful.

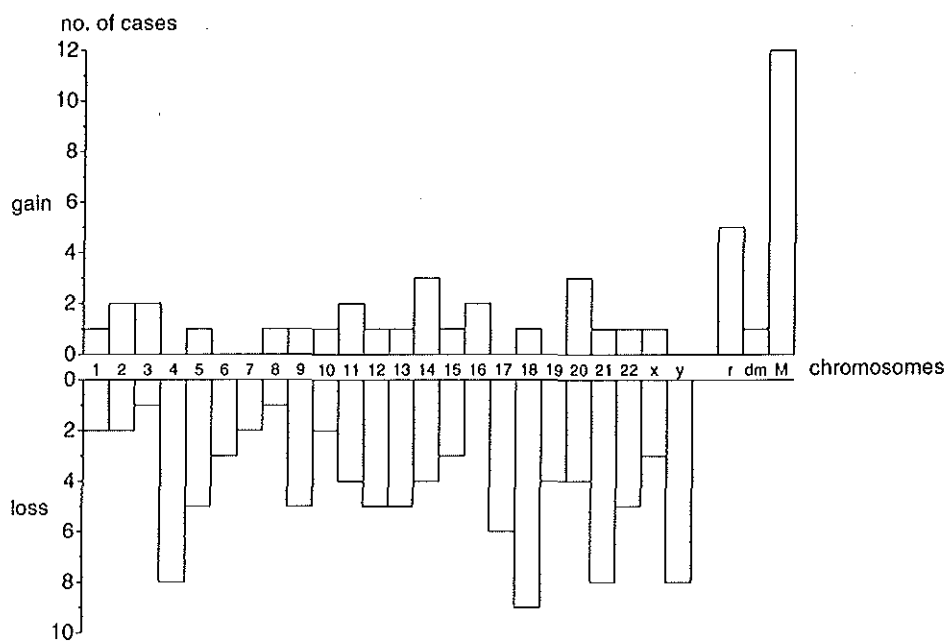
The results of flowcytometric and cytogenetic analysis analysis of adenocarcinoma located in the distal oesophagus without Barrett's mucosa and cardia are listed in table 5 and 6 respectively. FCM showed aneuploidy in 6 of the 10 adenocarcinomas of the distal oesophagus, while two were diploid and two were not investigated (table 5). In two of these (no.7 and 10) the DNA-index correlated with the modal chromosome number found at cytogenetic analysis. A FCM histogram of patient no. 7 showed a hypodiploid shoulder and an aneuploid peak with a DNA-index of 1.65 (Figure 1). Furthermore, three others had DNA-indices varying between 1.53 and 2.17, but hypodiploid cytogenetic clones were found (no.6,16 and 28). An inadequate number of metaphases was analyzed in 3 cases, and no abnormal clones were found, although FCM aneuploidy was seen in one. At least 5 metaphases were analyzed in 7 cases and clonal abnormalities were found in all of them. FCM showed a tetraploid population in one case and aneuploidy in another case of adenocarcinoma of the cardia (table 6). In both cases only one metaphase was analyzed and showed a normal karyotype. More than 10 metaphases were analyzed in one case, in which an abnormal clone was found. Constitutional chromosomal abnormalities were not found in any of the patients by cytogenetic analysis of periferal blood lymphocytes.

Figure 1. Histogram of flowcytometric analysis of tumour tissue of patient no 7.



Clonal chromosomal abnormalities in Barrett's mucosa were seen in two cases with near-diploid and near-triploid chromosome numbers respectively. In 19 cases clonal chromosomal abnormalities were found in tumour tissue. These were analyzed for the presence of common numerical and structural aberrations. All chromosomes contributed to numerical changes. Gains and losses of chromosomes, determined relatively to ploidy, were plotted on a histogram (figure 2).

Figure 2. Histogram of all clonal numerical chromosome changes observed in tumour cells of 19 patients with an adenocarcinoma in Barrett's oesophagus (11), the distal oesophagus without Barrett's mucosa (7) and cardia (1). Gains and losses depicted are determined relative to ploidy. Unidentified markers are also shown: r=ring chromosome, dm=double minute, mar=marker chromosome.



When compared to the ploidy of individual tumours whole chromosome losses were more common than gains. Losses of chromosomes 4, 18, 21 and Y were the most frequent numerical losses. Loss of the Y was observed in 8 of 13 tumours of males (62%).

Chromosomes 14 and 20 were most frequently gained. Loss of chromosome 16 was not observed. Structural abnormalities were observed in 13 of the abnormal clonal karyotypes (68%). All chromosome arms except 10p, 16q, 18q, 19p, 20q and chromosome X participated in structural changes (figure 3). The chromosome arms most frequently rearranged were 1p, 3q, 11p and 22p. Abnormalities of chromosome 1p arm frequently resulted in (partial) deletion, with the shortest region of overlap being at 1p22-33 (figure 4). In contrast, chromosome 11p and 22p rearrangements often showed gain of material. A homogenous staining region (hsr) of 11p14 was observed in 3 cases and gain at 11p15 in one case, whereas a deletion on chromosome arm 11p was seen in one case with the breakpoint at 11p14 (figure 4). Structural rearrangements of chromosome arm 3q were frequently observed, including deletions in 2 cases, gains in 2 cases, an unbalanced translocation in one case and isochromosomes in 3 cases (figure 4). The breakpoint occurred at the centromere in 4 cases. Several recurrent isochromosomes have been identified. The most frequent were i(3q) in 3 cases, i(13q) in 2 cases and i(14q) in 2 cases.

Figure 3. Histogram of all clonal structural chromosome changes observed in tumour cells of 12 patients with an adenocarcinoma in Barrett's oesophagus (8), the distal oesophagus without Barrett's mucosa (3) and cardia (1).
/// isochromosomes, XXX unbalanced translocations

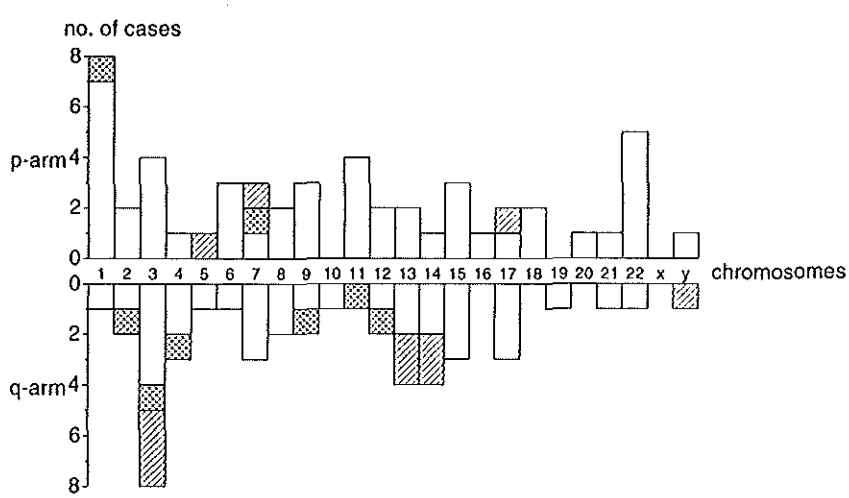


Figure 4. Examples of rearrangements affecting chromosome arms 1p, 3q and 11p in the studied tumours. Patient numbers are indicated under the rearranged chromosomes. R-bands are used in the majority of cases and Q-bands for patientno. 1 (chromosome 11), 5 (chromosome 1), 31 (chromosomes 1 and 3) and 36 (chromosome 3).

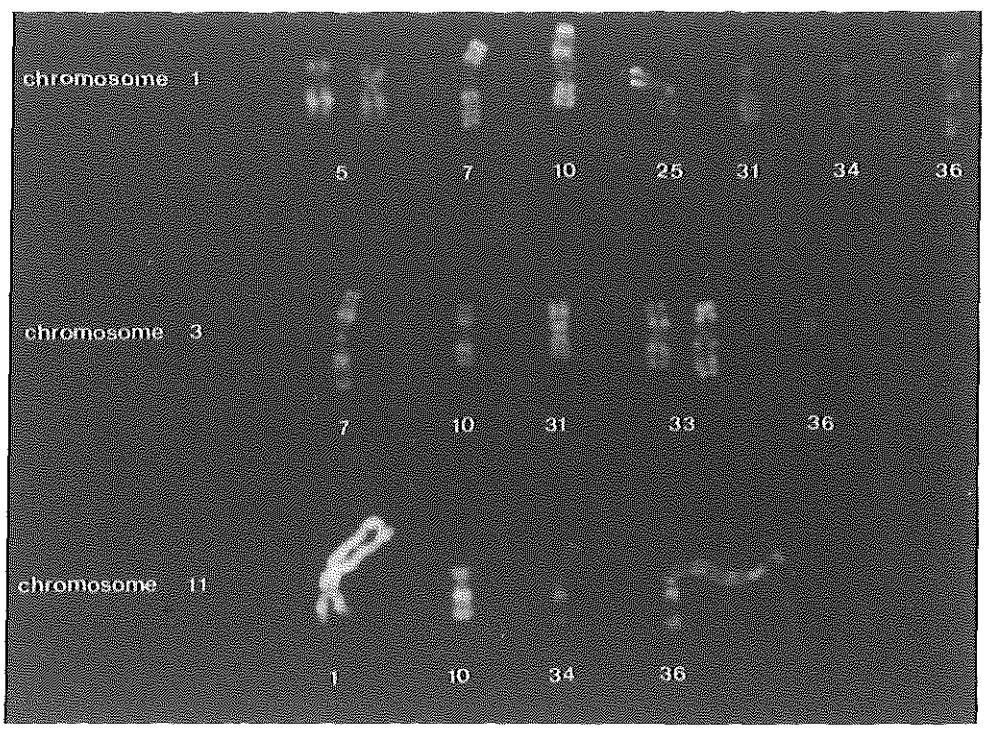


Table 1 CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF 22 PATIENTS WITH AN ADENOCARCINOMA IN BARRETT'S OESOPHAGUS

			ADENOCARCINOMA [1]		MUCOSA [1]	
PATIENT NO	SEX	AGE	pTNM	GRADE [2]	TYPE [3]	GRADE OF DYSPLASIA
1	F	76	T3N1M0	G1	–	–
3	M	61	T1N0M0	G3	–	–
5	M	58	T3N1M0	G2	SQUAMOUS	–
9	M	45	T2N0M0	G3	BARRETT	MILD
11	M	63	T1N0M0	G3	BARRETT	MILD
12	F	65	T3N1M0	G3	–	–
13	M	59	T3N1M0	G3	SQUAMOUS	–
17	M	80	T1N0M0	G2	BARRETT	MODERATE
18	M	67	T3N1M0	G3	–	–
19	M	69	T3N1M0	G3 (4)	BARRETT	NO
20	F	67	T3N1M1	G3	BARRETT	MODERATE
23	F	77	T3N0M0	G2	BARRETT	NO
24	F	68	T1N0M0	G2	BARRETT	SEVERE
25	F	75	T1N0M0	G2	BARRETT	MILD
26	F	67	T3N1M1	G3	–	–
27	M	77	T3N1M0	G3	BARRETT	NO
29	M	70	T2N0M0	G2	BARRETT	MILD
31	M	53	T3N1M0	G2	SQUAMOUS	–
32	F	64	T2N0M0	G1	BARRETT	NO
36	F	73	T3N1M1	G2	BARRETT	MODERATE
38	M	50	T3N1M0	G3	–	–
39	M	70	T3N0M0	G3	–	–

1. HISTOPATHOLOGICAL DIAGNOSIS OF TISSUE SAMPLES FOR FLOWCYTOMETRIC AND CYTOGENETIC ANALYSIS
2. DIFFERENTIATION GRADE: G1=WELL DIFFERENTIATED G2= MODERATELY DIFFERENTIATED G3=POORLY DIFFERENTIATED
3. HISTOLOGICAL DIAGNOSIS OF MUCOSA SPECIMENS ON WHICH CYTOGENETIC AND FLOWCYTOMETRIC ANALYSIS WERE PERFORMED
4. TISSUE SAMPLE FROM A LYMPH NODE METASTASIS

Table 2 FCM AND CYTOGENETIC ANALYSIS OF BARRETT'S MUCOSA

BARRETT'S MUCOSA						
PATIENT NO	FLOWCYTOMETRY (DNA-INDICES)	NUMBER OF METAPHASES ANALYZED				
		TOTAL	NORMAL	ABNORMAL NON-CLONAL	ABNORMAL CLONAL	KARYOTYPE OF CLONAL ABNORMALITY
9	ANEUPLOID (1.93,3.43)	–	–	–	–	–
11	DIPLOID	1	–	1	–	–
17	DIPLOID	16	7	4	5	45(34-46),X,-Y[cp5]
19	DIPLOID	26	20	6	–	–
20	TETRAPLOID	6	4	2	–	–
23	DIPLOID	2	2	–	–	–
24	ANEUPLOID (1.18,2.32)	3	2	1	–	–
25	ANEUPLOID (1.61)	5	–	–	1 + 4 [1]	72<3n>,XXX,+3,-4,-5,-5,-6,+8,+add(9)(p22),+del(10)(q23),-11,+12,-14,add(15)(p12),+add(15)(q21),add(17)(p12),-18,+add(19)(q13),+20,-21,+22,+mar1,+mar2[cp4]
27	DIPLOID	1	1	–	–	–
29	DIPLOID	–	–	–	–	–
32	DIPLOID	–	–	–	–	–
36	DIPLOID	–	–	–	–	–

1. INDICATES METAPHASES THAT WERE COUNTED, WITH MARKERS IDENTIFIED, BUT WHICH COULD NOT BE COMPLETELY KARYOTYPED

Table 3 FCM AND CYTOGENETIC ANALYSIS OF ADENOCARCINOMA IN BARRETT'S OESOPHAGUS

ADENOCARCINOMA IN BARRETT'S OESOPHAGUS						
PATIENT NO	FLOWCYTOMETRY (DNA-INDICES)	NUMBER OF METAPHASES ANALYZED				
		TOTAL	NORMAL	ABNORMAL NON-CLONAL	ABNORMAL CLONAL	KARYOTYPE OF CLONAL ABNORMALITY
1	–	10	–	–	1 6 + 4	39-42<2n>,X,-X,+3,-4,add(10)(q26),+hsr(11)(p14),-13,-17,-18,-20,-21, add(22)(p11),+mar,+dmin[cp10]
3	–	1	1	–	–	–
5	–	14	9	2	1 1 + 2	80<4n>,XXX,-Y,-Y,del(1)(p21p34),+add(1)(p22),+inv(2)(q13q33),-3,-4,-5,-6 del(7)(q31),-7,-9,-9,-10,-10,-10,+11,add(12)(p12),i(13)(q10),-13,-13,-13,-14 add(15)(q26)x2,add(18)(p11),-18,-18,-21,-21,-21,add(22)(p12)x2,+r,+mar1x2, +mar2, +mar3
9	ANEUPLOID (1.85,3.29)	9	6	3	–	–
11	TETRAPLOID	6	4	2	–	–
12	ANEUPLOID (1.65)	20	16	2	2	46-47,XX,+2mar[cp2]
13	DIPLOID	28	3	–	1 8 + 7	37-42<2n>,Xi(Y)(q10),del(1)(p21p34),+add(3)(q27),-5,i(7)(p10),add(8)(q24),-9,-11,-12,-13 i(13)(q10),-15,-19,-20,-22,add(22)(q13),+r,+mar [cp15]
					1 7 + 3	69(64-83)<3n>,XXi(Y)(q10),+2,+3,+add(3)(q27),+4,-5,+add(6)(q26)x2,i(7)(p10),+i(7)(p10) add(8)(q24)x2,-10,-11,-12,i(13)(q10)x2,+14,+15,-17,-18,-19,-20,+21,add(22)(q13)x2, +rx2,+mar[cp10]
17	ANEUPLOID (1.56)	34	12	6	16	45(34-45),X,-Y[10],-10,-14,-17,-18,-21 [cp16]
18	ANEUPLOID (1.49)	37	17	2	18	45(36-47),X,-Y[9],-2,-6,-7,-9,-11,-18,-19,-22 [cp18]
19	ANEUPLOID (1.50,2.05)	11	5	2	4	66-69<3n>,X,-Y,add(9)(p22),+mar [cp4]

Table 3 (continued)

PATIENT NO	FLOWCYTOMETRY (DNA-INDICES)	NUMBER OF METAPHASES ANALYZED				KARYOTYPE OF CLONAL ABNORMALITY
		TOTAL	NORMAL	ABNORMAL NON-CLONAL	ABNORMAL CLONAL	
20	DIPLOID	8	6	2	–	–
23	ANEUPLOID (1.98, 2.93)	12	9	3	–	–
24	ANEUPLOID (1.20, 2.43)	6	4	2	–	–
25	ANEUPLOID (1.61)	6	–	–	4 + 2[1]	63-71<3n>,XXX,+X,del(1)(p21p33),+3,-4,-5,+8,add(9)(p22),+10,-13,+14,add(15)(p12),add(15)(q21),-18,+add(19)(q13),add(21)(p21),+22,+mar1,+mar2[cp6]
26	ANEUPLOID (1.47, 1.78)	4	4	–	–	–
27	ANEUPLOID (1.49, 2.07)	–	–	–	–	–
29	ANEUPLOID (1.69)	8	6	2	–	–
31	ANEUPLOID (1.42)	7	1	2	2 + 2[1]	51-55<2n>,Xadd(Y)(p13),+del(1)(p22),+del(2)(p15),+add(3)(p26),+del(3)(q24),+9,+11,+add(14)(q32),hsr(14)(p11),i(17)(p10)x2,add(18)(p11),+20,+20,-21,-22,-22,+mar [cp4]
32	ANEUPLOID (1.99, 2.79)	3	2	1	–	–
36	ANEUPLOID (1.72)	8	–	–	5 + 3[1]	76(61-95)<3n>,XX,-X,+1,+del(1)(p22),+del(2)(p13),+der(2;3)(q10;q10),+del(3)(q21),-4,add(4)(q27),+der(4;12)(q10;q10),add(6)(p22),del(7)(q31)x2,-8,der(9)t(9;10)(q34;q22)x2,+hsr(11)(p14),-12,+13,der(15)t(1;15)(p22;p13)x3,-17,+20,+20,+20,+r,+mar1,+mar2,+mar3[cp8]
38	–	1	1	–	–	–
39	–	23	10	8	5	39-45<2n>,XY,-19,-21 [cp5]

1. INDICATES METAPHASES THAT WERE COUNTED, WITH MARKERS IDENTIFIED, BUT WHICH COULD NOT BE COMPLETELY KARYOTYPED

Table 4 CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF PATIENTS WITH AN ADENOCARCINOMA IN THE DISTAL OESOPHAGUS WITHOUT BARRETT'S MUCOSA (DO) OR CARDIA (C)

			ADENOCARCINOMA		
PATIENT NO	SEX	AGE	pTNM	GRADE [1]	LOCALIZATION
2	M	44	T3N1M1	G3	D0
6	M	68	T3N1M0	G2	D0
7	M	46	–	G2 (2)	D0
8	M	44	–	G2 (2)	D0
10	F	62	T3N1M0	G3	D0
16	M	63	T2N1M1	G3	D0
28	M	44	T2N0M0	G2	D0
34	M	54	T3N1M0	G2	D0
35	M	68	T3N1M0	G3	D0
37	M	53	T2N1M0	G3	D0
14	M	69	T3N2M0	G3	C
21	M	64	T3N1M0	G3	C
22	M	41	T3N1M0	G3	C
30	M	63	T3N2M1	G3	C
33	F	73	T3N2M0	G2	C

1. DIFFERENTIATION GRADE: G1=WELL DIFFERENTIATED
G2 =MODERATELY DIFFERENTIATED
G3 = POORLY DIFFERENTIATED

2. TISSUE SAMPLE FROM A LYMPH NODE METASTASIS

Table 5 FCM AND CYTOGENETIC ANALYSIS OF ADENOCARCINOMA IN THE DISTAL OESOPHAGUS WITHOUT BARRETT'S MUCOSA

PATIENT NO	FLOWCYTOMETRY (DNA–INDICES)	NUMBER OF METAPHASES ANALYZED				KARYOTYPE OF CLONAL ABNORMALITY
		TOTAL	NORMAL	ABNORMAL NON–CLONAL	ABNORMAL CLONAL	
2	–	1	1	–	–	–
6	ANEUPLOID (1.53)	5	2	–	3 1	45(45–64)<2n>,X,–Y[cp3]
7	ANEUPLOID (1.65)	21	–	–	12 + 9	63–68<3n>,XXYY,+der(1)t(1;9)(p12;q12),+der(1)t(1;9)(p12;q12),+2,i(3)(q10)x2,add(3)(p26),add(4)(q23),–1,–12,der(12)t(10;13)(q23;p12)x2,del(13)(q12),+del(13)(q12),+del(13)(q12),+14,+15,der(16)t(13;16)(q12,p13)+der(16)t(13;16)(q12;p13),–17,–18,–19,–20,add(21)(q21)x2,–21,del(22)(p11)x2,der(22)t(3;22)(p12;p13),+der(22)t(3:22)(p12;p13),+r,+mar [cp21]
8	DIPLOID	17	10	2	5 1	91–94<4n>,XXYY,+12,+18 [cp5]
10	ANEUPLOID (1.56, 1.94)	23	2	–	5 + 16	50–64<2n>,XX,add(1)(p33),+add(1)(q43),+2,del(3)(p14),+i(3)(q10),del(4)(p12),–4,add(5)(q35),+5,add(6)(p23),del(7)(q31),+der(7;11)(p10;q10),del(8)(p21),del(11)(p14),add(11)(p15),+hsr(12)(p12),add(13)(p11),–13,i(14)(q10),add(15)(q26),+16,del(17)(q25),+add(17)(p12),+20,+20,+21,+21,add(22)(p12),+mar[cp21]
16	ANEUPLOID (1.69)	32	19	2	11	40(28–45)<2n>,X,–Y[5],–1,–2,–4,–5,–9,–11,–13,–17,–18,–22 [cp11]
28	ANEUPLOID (2.17)	16	12	1	3 1	44–45,X,–Y[cp3]
34	–	8	–	–	4 + 4	58–60<3n>,XX,–Y,add(1)(p11),del(3)(p24),+del(3)(p24),–4,i(5)(p10),–6,add(7)(p17)add(8)(q24)x2,add(9)(p21),–9,hsr(11)(p14),del(12)(q21),–12,i(14)(q10),–14,–15,–17,–17,–18,–18,–18,–21,–21,+r,+4mar [cp8]
35	ANEUPLOID (1.73)	1	1	–	–	–
37	DIPLOID	1	1	–	–	–

1. INDICATES METAPHASES THAT WERE COUNTED, WITH MARKERS IDENTIFIED, BUT WHICH COULD NOT BE COMPLETELY KARYOTYPED

Table 6 FCM AND CYTOGENETIC ANALYSIS OF ADENOCARCINOMA IN THE CARDIA

PATIENT NO	FLOWCYTOMETRY (DNA-INDICES)	NUMBER OF METAPHASES ANALYZED				
		TOTAL	NORMAL	ABNORMAL NON-CLONAL	ABNORMAL CLONAL	KARYOTYPE OF CLONAL ABNORMALITY
14	–	6	6	–	–	–
21	TETRAPLOID	1	1	–	–	–
22	ANEUPLOID (1.41, 2.69)	1	1	–	–	–
30	–	6	5	1	–	–
33	–	11	2	–	9	60-77<3n>,XX,-X,-1,add(3)(q29),+i(3)(q10),-4,-5,del(6)(q24)x2,+add(6)(p22),add(8)(p22),-12,add(13)(q34),+add(13)(q34),add(14)(q32)x2,-14,add(15)(p13),-15,+16,+16,+16,+16,add(17)(q25),del(17)(q22),-18,add(20)(p11),-20,-21,-22,+mar [cp9]

Discussion

In 1976 Nowell hypothesized that cancer develops in association with an acquired genetic instability that predisposes to the acquisition of abnormal clones of cells with accumulated genetic errors (19). It has been demonstrated that neoplastic progression in Barrett's oesophagus is associated with a similar process of clonal evolution (8,9,13,20). In our study we analyzed 37 adenocarcinomas of the oesophagus and cardia of which 22 arose in Barrett's oesophagus. In Barrett's mucosa we found normal karyotypes, except in one case where a complex karyotype was found with the same markers and rearrangements as in the adenocarcinoma of the same patient. A theoretical possibility in this case is that the abnormal tissue obtained from the Barrett's segment that was spatially separate from the cancer contained an unidentified microscopic focus of carcinoma. A specific karyotypic change common to all cases with genetic abnormalities was not found. The absence of differences in the complex pattern of cytogenetic changes between adeno-carcinomas in Barrett's oesophagus, the distal oesophagus without Barrett's mucosa or cardia suggests a common pathway of origin at all three anatomical sites. This is similar to the results of others (11,12). A good correlation between DNA-indices of the aneuploid tumours and the modal chromosome numbers of the abnormal karyotypes was found in most of the tumours with multiple and complex chromosomal rearrangements. Recurrent combinations of hypodiploid and triploid clones correspond to a pattern of clonal progression that is characterized by chromosome losses followed by secondary duplication and losses. In cases with a few numerical changes there was discordance between DNA-indices and modal chromosome numbers of the karyotypes. DNA-content flowcytometry can determine the relative proportions of populations of different ploidies, but may not detect near-diploid or minority populations. By cytogenetic analysis it is possible to identify a clone, even when it comprises a small number of cells, but it cannot estimate its prevalence. Populations of cells with a single or a few numerical changes will be near-diploid and are therefore seldom detected by flowcytometry. On the other hand, DNA-aneuploid clones found by flowcytometry may not be analyzed cytogenetically due to tissue selection. Whereas balanced translocations were seldom found in this study, losses due to missing chromosomes or apparently unbalanced rearrangements (derivative chromosomes, deletions and isochromosomes) were frequently observed. Marker chromosomes were common and some of the missing chromosomal

material obviously resides in these unidentified chromosomes. Whole chromosome losses of 4, 18, 21 and in males Y were the most frequent numerical changes in this series. Frequent numerical loss of chromosomes 4, 18, 21 and Y has been described in colorectal carcinoma (21-24). Loss of the Y-chromosome has been demonstrated in different gastrointestinal malignancies (21,22,25) and brain tumours (24,26). In Barrett's oesophagus and oesophageal adenocarcinoma loss of the Y-chromosome also appears to be a common occurrence (10,11,20,27). It has been suggested that tissues retaining rapid cellular proliferation rates in elderly men are more prone to loss of the Y-chromosome (28). In our series loss of the Y-chromosome in Barrett's mucosa and adenocarcinoma occurred in 31% of the male patients, but not merely the oldest ones. Furthermore, cytogenetic analysis of peripheral blood lymphocytes did not reveal Y-loss. Although there is a male predominance in the incidence of Barrett's oesophagus and oesophageal adenocarcinoma (1,4,5,29), the significance of Y-loss is unclear since there are no cancer genes linked to the Y-chromosome so far. Recurrent gains of chromosomes 14 and 20 were seen in this series. Extra copies of chromosome 20 were also found in colorectal carcinomas (21,24). Structural abnormalities of chromosome arms 1p, 3q, 11p and 22p were most frequently observed. Structural rearrangements of the short arm of chromosome 1 resulted most often in deletions with the shortest region of overlap being 1p22-33. Frequent rearrangements of chromosome arm 1p were also described in gastric and colorectal carcinomas (22,25). N-ras, a transforming gene from a neuroblastoma cell line has been assigned to 1p22-31 (30,31). Rao et al. described structural rearrangements of 3q in 6 of 9 gastric and oesophageal adenocarcinomas (12). Allelotype analysis in osteosarcomas suggests the existence of tumour suppressor genes on the long arm of chromosome 3 (32). Structural rearrangements in the 11p13-15 region were found in Barrett's adenocarcinomas and other adenocarcinomas of the oesophagus and stomach (11). Genetic changes affecting the 11p13-15 region have previously been associated with Wilm's tumour, hepatoblastoma, rhabdomyosarcoma, carcinoma of the bladder and breast (33-35). Furthermore, the H-ras-1 protooncogene has been localized to band 11p15 (35). Whether these cancer genes are implicated in the progression of oesophageal tumours should be investigated using molecular techniques.

These studies show that Barrett's mucosa and adenocarcinoma can be successfully karyotyped using banding techniques. Clonal evolution characterized by chromosome loss and further duplication of the hypodiploid karyotype is a known mechanism in adenocarcinoma

and has previously been described, particularly in coloncarcinoma (36). A chromosomal abnormality common to all tumours was not identified, although hot spots for structural rearrangements have been seen in chromosome 1p, 3q, 11p and 22p as well as frequent losses of chromosomes 4, 18, 21 and Y. Further studies should include the use of molecular cytogenetic methodology, in particular fluorescent in situ hybridization with chromosome specific probes and comparative genomic hybridization. In addition loss of heterozygozity analyses for chromosome loss or deletions and molecular investigations of cancer genes possibly involved, e.g. the ras genes and various tumour suppressor genes seem already indicated.

References

1. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. Gastroenterology 1993; 104: 510-513.
2. Powell J, McConkey CL. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. Br J Cancer 1990;59:440-443.
3. DeMeester TR. Barrett's esophagus. Surgery 1993; 113: 239-241.
4. Williamson WA, Ellis FH, Gibb SP, et al. Barrett's esophagus: prevalence and incidence of adenocarcinoma. Arch Int Med 1991; 151: 2212-2216.
5. Menke-Pluymers MBE, Schoute NW, Mulder AH, Hop WCJ, van Blankenstein M, Tilanus HW. Outcome of surgical treatment of adenocarcinoma in Barrett's oesophagus. Gut 1992; 33: 1454-1458.
6. Streitz JM, Ellis FH, Gibb SP, Balogh K, Watkins E. Adenocarcinoma in Barrett's esophagus. A clinicopathologic study of 65 cases. Ann Surg 1991; 213: 122-125.
7. Van der Veen AH, Dees J, Blankensteyn JD, van Blankenstein M. Adenocarcinoma in Barrett's oesophagus: an overrated risk. Gut 1989; 30: 14-18.
8. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett's esophagus is associated with genomic instability. Lab Invest 1989; 60: 65-71.
9. Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC, Rabinovitch PS. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenterology 1992; 102: 1212-1219.
10. Garewal HS, Sampliner R, Liu Y, Trent JM. Chromosomal rearrangements in Barrett's esophagus. A premalignant lesion of esophageal adenocarcinoma. Cancer Genet Cytogenet 1989; 42: 281-296.
11. Rodriguez E, Rao PH, Ladanyi M, Altorki N, Albino AP, Kelsen DP, et al. 11p13-15 Is a specific region of chromosomal rearrangement in gastric and esophageal adenocarcinomas. Cancer Res 1990; 50: 6410-6416.

12. Rao PH, Mathew S, Kelsen DP, Chaganti RSK. Cytogenetics of gastric and esophageal adenocarcinomas. 3q Deletion as a possible primary chromosomal change. Cancer Genet Cytogenet 1995; 81: 139-143.

13. Raskind WH, Norwood T, Levine DS, Haggitt RC, Rabinovitch PS, Reid BJ. Persistent clonal areas and clonal expansion in Barrett's esophagus. Cancer Res 1992; 52: 2946-2950.

14. Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol 1980; 33: 711-721.

15. Vindelov LL, Christensen IJ, Nissen NI. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 1983;3:323-327.

16. Hiddeman W, Sluman J, Andreeff M, Barlogie B, Herman ChJ, Leif RC, et al. Special report. Convention on nomenclature for DNA-cytometry. Cytometry 1984;5:445-446.

17. Hagemeijer A, Smit EME, Bootsma D. Improved identification of chromosomes of leucemic cells in methotrexate treated cultures. Cytogenet Cell Genet 1979;23:208-212.

18. ISCN (1991). Guidelines for Cancer Cytogenetics, Supplement to an International System for Human Cytogenetic Nomenclature, F Mitelman (ed); S Karger, Basel.

19. Nowell PC. The clonal evolution of tumor cell populations. Science 1976; 194: 23-28.

20. Krishnadath KK, Tilanus HW, van Blankenstein M, Hop WCJ, Teygeman R, Mulder AH, Bosman FT, van Dekken H. A cumulation of genetic abnormalities during neoplastic progression in Barrett's esophagus. Cancer Res 1995; 55: 1971-1976.

21. Bardi G, Johansson B, Pandis N, Mandahl N, Bak-Jensen E, Fenger C. Cytogenetic findings in 152 colorectal adenocarcinomas - correlation with clinicopathologic features. 4th European workshop on cytogenetics and molecular genetics of human solid tumors (Abstract).

22. Xiao S, Wei W, Feng XL, Shi YH, Liu QZ, Li P. Direct chromosome analysis of seven primary colorectal carcinomas. Cancer Genet Cytogenet 1992; 62: 32-39.

23. Muleris M, Salmon RJ, Dutrillaux B. Cytogenetics of colorectal adenocarcinoma. Cancer genet Cytogenet 1990; 46: 143-156.

24. Mitelman F. Catalogue of chromosome aberrations in cancer, 1990 3rd edition. Ed. Alan R Liss, New York.

25. Ochi H, Douglass HO, Sandberg A. Cytogenetic studies in primary gastric cancer. Cancer Genet Cytogenet 1986; 22: 295-307.

26. Bigner SH, Mark J, Bullard DE, Mahaley MS, Bigner DD. Chromosomal evolution in malignant human gliomas starts with specific and usually numerical deviations. Cancer Genet Cytogenet 1986; 22: 121-135.

27. Hunter S, Gramlich T, Abbott K, Varma VY. Chromosome loss in esophageal carcinoma; an in situ hybridization study. Genes Chrom Cancer 1993; 8: 172-177.

28. Pierre RV, Hoagland HC. Age associated aneuploidy: loss of Y-chromosome from human bone marrow cells with aging. Cancer 1972; 30: 889-894.

29. Menke-Pluymers MBE, Hop WCJ, Dees J, van Blankenstein M, Tilanus HW. Risk factors for the development of an adenocarcinoma in columnar-lined (Barrett) esophagus. Cancer 1993; 72: 1155-1158.

30. de Martinville B, Cunningham JM, Murray MJ, Francke U. The N-ras oncogene assigned to chromosome 1(p31$^{?}$->cen) by somatic cell hybrid analysis. Cytogenet Cell Genet 1984; 37: 531.

31. Davis M, Malcolm S, Hall A. The N-ras oncogene is located on the short arm of chromosome 1. Cytogenet Cell Genet 1984; 37: 448.

32. Yamaguchi T, Toguchida J, Yamamura T, Kotoura Y, Takada N, Kawaguchi N, et al. Allelotype analysis in osteosarcomas: Frequent allele loss on 3q, 13q, 17p and 18q. Cancer Res 1993; 52: 2419-2423.

33. Fearon ER, Feinberg AP, Hamilton SH, Vogelstein B. Loss of genes on the short arm of chromosome 11 in bladder cancer. Nature (London) 1985; 318: 377-380.

34. Theillet C, Lidereau R, Escot C, Hutzell P, Brunet M, Gest J, Schlom J, Callahan R. Loss of a c-H-ras-1 allele and aggressive human primary breast carcinomas. Cancer Res 1986; 46: 4776-4781.

35. Popescu NC, Amsbaugh SC, Di Paolo JA, Tronick SR, Aaronson SA Swan DC. Chromosomal localization of three human ras genes by in situ molecular hybridization. Somatic Cell Mol Genet 1985; 11: 149-155.

36. Remvikos Y, Muleris M, Vielh Ph, Salmon RJ, Dutrillaux B. DNA content and genetic evolution of human colorectal adenocarcinoma. A study by flow cytometry and cytogenetic analysis. Int J Canc 1988;42:539-543.

CHAPTER 6

General discussion and conclusions

There has been an increase in the incidence of adenocarcinoma of the oesophagus and the oesophagogastric junction in North America and Western Europe in recent years, whereas rates of squamous cell carcinoma of the oesophagus have remained fairly constant (1,2). Prognosis for patients with an oesophageal carcinoma, both adenocarcinoma and squamous cell carcinoma, is poor, even after oesophagectomy (3-6). Once a carcinoma has developed and leads to symptoms, the tumour is often in an advanced stage and survival is poor. In contrast to squamous cell carcinoma of the oesophagus, adenocarcinoma has a precursor lesion; patients with Barrett's oesophagus run a 30 to 125 fold increased risk of developing an oesophageal adenocarcinoma (7-9).

As demonstrated in chapter 2.1 prognosis of patients with adenocarcinoma in Barrett's oesophagus is influenced by oesophageal wall penetration and the presence of lymph node metastases: patients with a tumour restricted to the submucosa without lymph node metastases had the best survival rates.

In chapter 2.2 we showed a relation between DNA ploidy of tumour cells and survival. Although the number of patients in this study was too small for reliable statistic analysis, there is an indication that increasing genomic instability of tumour cells leading to aneuploidy is related to poor survival.

It can be concluded from these studies that early diagnosis will improve the prognosis of patients with an adenocarcinoma in Barrett's oesophagus.

Since early diagnosis of oesophageal adenocarcinoma will benefit the patient, endoscopic surveillance programs have been developed for patients with Barrett's oesophagus, although there is no agreement about the intensity of such a screening programme (9,10-12). Many authors have stressed the importance of annual endoscopic screening, but others suggested that only patients at high risk of developing invasive carcinoma should be screened (11-15). To create more efficient surveillance programs, it is necessary to define factors which indicate increased risk of malignant change for selecting a subgroup of patients at high risk.

One of the factors indicating high risk of malignant change is the extent of Barrett's mucosa. Although oesophageal adenocarcinoma can develop in short segments of Barrett's mucosa, patients with an extensive columnar lined segment have an increased

risk of malignancy (12,16,17). In chapter 3 we demonstrated that a doubling of the length of a columnar lined segment resulted in about a doubling of the risk of developing carcinoma. Columnar lined segments of 6 cm or more were more common in patients with adenocarcinoma in Barrett's oesophagus compared to patients with benign Barrett's oesophagus. In the literature Barrett's patients with a columnar lined segment of 8 cm or more are regarded to be at particular risk (12,16,17).

There is a male predominance among patients with Barrett's oesophagus and even a higher male to female ratio in the group with adenocarcinoma in Barrett's oesophagus.

In accordance with several studies in the literature we identified a correlation between smoking and adenocarcinoma in Barrett's oesophagus, but not between alcohol, consumption and carcinoma (18,19).

It can be concluded that male patients, patients with a columnar lined segment of 6 cm or more and smokers form a subgroup at risk for neoplastic progression in Barrett's oesophagus. Therefore, it can be considered to exclude female patients with a columnar lined segment less than 6 cm, who do not smoke from the screening programme, or to screen these patients at longer intervals.

Dysplasia in columnar lined oesophagus is the most important indicator of impending malignant change and at present the only reliable marker (20-22). In the literature a strong association between high grade dysplasia and adenocarcinoma in Barrett's oesophagus has been demonstrated, so that patients with high grade dysplasia should be offered oesophageal resection or at least frequent endoscopies with extensive biopsies (20-24). As demonstrated in chapter 4, we also found an increased risk of malignancy in Barrett's oesophagus if dysplasia was present, especially moderate or severe dysplasia. However, flowcytometric analysis revealed that DNA-ploidy was not statistically correlated with dysplasia. The relationship between DNA-ploidy and dysplasia is controversial. Although some authors showed that flowcytometric abnormalities correlated well with dysplastic changes, others found that aneuploidy and dysplasia were often discordant (25,26). We found that dysplasia and aneuploidy or increased G2/tetraploidy were independent risk factors for malignant degeneration in Barrett's oesophagus. Data from prospective follow-up studies indicated that aneuploidy and dysplasia were both markers for the subsequent development of adenocarcinoma in

Barrett's oesophagus (27,28). Although it has been demonstrated that none of the patients with flowcytometric abnormalities progressed to invasive cancer without first exhibiting high grade dysplasia, flowcytometry may help identifying patients at risk for developing high grade dysplasia or invasive carcinoma. On the basis of our finding that both dysplasia and aneuploidy or increased G2/tetraploidy are independent risk factors for malignant degeneration in Barrett's oesophagus, patients with either or both these risk factors should be offered at least annual or half yearly screening, provided that they are able to undergo surgical intervention. Patients without these factors may be screened at greater intervals guided by other risk factors.

Cancer develops in association with an acquired genetic instability that predisposes to the acquisition of abnormal clones of cells with accumulated genetic errors (29). Neoplastic progression in Barrett's oesophagus is associated with a similar process of clonal evolution (25,27,30,31). Rabinovitch et al. found cell populations with single common abnormal DNA contents extending over large areas of dysplastic mucosa, suggesting that dysplastic epithelium arises by expansion of a single clone of genetically altered cells (25). The findings of Raskind et al. suggested that neoplastic progression in Barrett's oesophagus involves the development of abnormal clones that can expand to occupy extensive regions of oesophageal mucosa, persist for several years, and progress to high grade dysplasia and adenocarcinoma (30). To discover clonal chromosomal rearrangements common to all oesophageal adenocarcinomas we performed cytogenetic analysis of adenocarcinoma of the distal oesophagus and cardia. Cytogenetic analysis was also performed on Barrett's mucosa to investigate if chromosomal abnormalities occurred as an early event before invasive tumour could be demonstrated.

Abnormal karyotypes with multiple and complex rearrangements were found. A chromosomal abnormality common to all tumours was not identified, although hot spots for structural rearrangements have been found in chromosome 1p, 3q, 11p and 22p as well as frequent losses of chromosomes 4, 18, 21 and Y. Other studies in Barrett's oesophagus demonstrated frequent loss of the Y chromosome, both in dysplastic mucosa and adenocarcinoma (31-33). Its significance is unclear since there are no cancer genes linked to the Y chromosome until now. Frequent losses of chromosomes 4, 18, 21 and Y have been described in colorectal carcinoma (34,35). So far, cytogenetic analysis of

Barrett's mucosa and adenocarcinoma is not useful in clinical practice.

There is a striking parallel between neoplastic progression in Barrett's mucosa and that in colonic epithelium. Specialized columnar epithelium and normal colonic mucosa share a common epithelial protein, suggesting a histogenetic relation between specialized Barrett's epithelium and colonic type epithelium (36). Dysplastic changes found in Barrett's oesophagus are similar to those found in inflammatory bowel disease, so that a grading system developed for inflammatory bowel disease can be used for Barrett's oesophagus (37). As in Barrett's mucosa, most carcinomas and areas of dysplasia appear to develop on the background of aneuploid mucosa (38). Also, genetic changes in Barrett's dysplastic mucosa and adenocarcinoma are similar to those in colonic mucosa. Losses of chromosomes 4, 18, 21 and Y, which we frequently found in adenocarcinomas of the distal oesophagus and cardia, have also been described in colorectal cancer (34,35). Blount et al. found that allelic losses of 17p occur as early events that precede the development of aneuploidy and other allelic losses during neoplastic progression in Barrett's oesophagus (39). Mutations and loss of the p53 gene are common events in ulcerative colitis associated dysplasia and carcinoma, and late events in sporadic colorectal tumourigenesis (40,41). Furthermore, allelic losses of chromosome 5q (APC, MCC) and 18q (DCC) have been found during neoplastic progression of both Barrett's mucosa and colorectal carcinoma (42,43).

Another striking finding was done by Sontag et al.; they found 19 benign and 10 malignant colon neoplasms in a series of 65 Barrett's patients (44).

Since the histopathological progression of colon cancer, commonly known as the adenoma to carcinoma sequence, now serves as a paradigm for the progression of most human cancers, the parallel between neoplastic progression in Barrett's oesophagus and colonic epithelium should be taken into account for future studies. These studies, using molecular cytogenetic techniques and losses of heterozygozity analysis, should be performed to identify one or more genetic changes that may provide rational markers for early cancer diagnosis.

References

1. Pera M, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. Gastroenterology 1993;104:510-513.
2. Powell J, McConkey CL. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. Br J Cancer 1990;59:440-443.
3. Survival plaveiselcelcarc.
4. Streitz JM, Ellis FH Jr, Gibb SP, Balogh K, Watkins E. Adenocarcinoma in Barrett's esophagus. A clinicopathological study of 65 cases. Ann Surg 1991;213:122-125.
5. Sanfey H, Hamilton SR, Smith RRL. Carcinoma arising in Barrett's esophagus. Surg, Gyn & Obstet 1985;161:570-574.
6. Lerut T, Coosemans W, Van Raemsdonck D, Dillemans B, De Leyn P, Marnette JM. Surgical treatment of Barrett's carcinoma. Correlation between morphologic findings and prognosis. J Thorac Cardiovasc Surg 1994;107:1059-1066.
7. Polepalle SC, McCallum RW. Barrett's esophagus: current assessment and future perspectives. Gastroenterol Clin North Am 1990;19:733-744.
8. Williamson WA, Ellis FH Jr, Gibb SP, et al. Barrett's esophagus: prevalence and incidence of adenocarcinoma. Arch Intern Med 1991;151:2212-2216.
9. Hameeteman W, Tytgat GNJ, Houthoff HJ, et al. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenetrology 1989;96:1249-1256.
10. Robertson CS, Mayberry JF, Nicholson DA, James PD, Atkinson M. Value of endoscopic surveillance in the detection of neoplastic change in Barrett's oesophagus. Br J Surg 1988;75:760-763.
11. Spechler SJ, Robbins AH, Bloomfield Rubins H, Vincent ME, Heeren T, et al. Adenocarcinoma and Barrett's oesophagus. An overrated risk? Gastroenterology 1984;87:927-933.
12. Van der Veen AH, Dees J, Blankensteyn JD, van Blankenstein M. Adenocarcinoma in Barrett's oesopahgus: an overrated risk. Gut 1989;30:14-18.

13. Streitz JM, Williamson WA, Ellis FH Jr. Current concepts concerning the nature and treatment of Barrett's esophagus and its complications. Ann Thorac Surg 1992;54:586-591.

14. Streitz JM, Andrews CW, Ellis FH. Endoscopic surveillance of Barrett's oesophagus. Does it help? J Thorac Cardiovasc Surg 1993;105:383-388.

15. Spechler SJ. Endoscopic surveillance for patients with Barrett esophagus: does the cancer risk justify the practice? Ann Int Med 1987;106:902-903.

16. Ransom JM, Patel GK, Clift SA, Wamble NE, Read RC. Extended and limited types of Barrett's esophagus in the adult. Ann Thorac Surg 1982;33:19-27.

17. Harle IA, Finley RJ, Belsheim M, Bondy DC, Booth M, Lloyd D, et al. Managemant of adenocarcinoma in a columnar-lined esophagus. Ann Thorac Surg 1985;40:330-336.

18. Skinner DB, Walther BC, Riddell RH, Schmidt H, Iascone C, DeMeester T. Barrett's esophagus: comparison of benign and malignant cases. Ann Surg 1983;198:554-566.

19. Gray MR, Kingsnorth AN. Smoking and cancer risk in Barrett's columnar lined oesophagus [abstract]. Gut 1990;31:AT56.

20. Reid BJ, Weinstein WM, Lewin KJ, Haggitt R, VanDeventer G, DenBesten L, et al. Endoscopic biopsy can detect high-grade dysplasia or early adenocarcinoma in Barrett's esophagus with or without grossly recognizable neoplastic lesions. Gastroenterology 1988;94:81-90.

21. Hamilton SR, Smith RR. The relationship between columnar epithelial dysplasia and invasive adenocarcinoma arising in Barrett's esophagus. Am J Clin Pathol 1987;87:301-312.

22. Levine DS, Haggitt RC, Blount PC, Rabinovitch PS, Rush VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993;105:40-50.

23. Pera M, Trastek VF, Carpenter HA, Allen MS, Deschamps C, Pairolero PC. Barrett's esophagus with high-grade dysplasia: an indication for esophagectomy? Ann Thorac Surg 1992;54:199-204.

24. Putnam JB, Soysal O, Roth JA, Walsh GL, Nesbitt JC, Schrump DS. results of esophagectomy for severe dysplasia in Barrett's esophagus. Sixth World Congress of the ISDE, 1995 (Abstract).

25. Rabinovitch PS, Reid BJ, Haggitt RC, Norwood TH, Rubin CE. Progression to cancer in Barrett's esophagus is associated with genomic instability. Lab Invest 1988;60:65-71.

26. Fennerty MB, Sampliner RE, Way D, Riddell R, Steinbronn K, Garewal HS. Discordance between flow cytometric abnormalities and dysplasia in Barrett's esophagus. Gastroenterology 1989;97:815-820.

27. Reid BJ, Blount PL, Rubin CE, Levine DS, Haggitt RC, Rabinovitch PS. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenterology 1992;102:1212-1219.

28. Garewal HS, Sampliner RE, Fennerty MB. Flow cytometry in Barrett's esophagus. What have we learned so far? Dig Dis Sci 1991;36:548-551.

30. Raskind WH, Norwood T, Levine DS, Haggitt RC, Rabinovitch PS, Reid BJ. Persistent clonal areas and clonal expansion in Barrett's esophagus. Cancer Res 1992;52:2946-2950.

31. Krishnadath KK, Tilanus HW, van Blankenstein M, Hop WCJ, Teygeman R, Mulder AH, Bosman FT, van Dekken H. A cumulation of genetic abnormalities during neoplastic progression in Barrett's esophagus. Cancer Res 1995;55:1971-1976.

32. Garewal HS, Sampliner R, Liu Y, trent JM. Chromosomal rearrangements in Barrett's esophagus. A premalignant lesion of esophageal adenocarcinoma. Cancer Genet Cytogenet 1989;42:281-296.

33. Hunter S, Gramlich T, Abbott K, Varma VY. Chromosome loss in esophageal carcinoma; an in situ hybridization study. Genes Chrom Cancer 1993;8:172-177.

34. Bardi G, Johansson B, Pandis N, Mandahl N, Bak-Jensen E, Fenger C. Cytogenetic findings in 152 colorectal adenocarcinomas - correlation with clinicopathologic features. 4th European workshop on cytogenetics and molecular genetics of human solid tumors (Abstract).

35. Muleris M, Salmon RJ, Dutrillaux B. Cytogenetics of colorectal adenocarcinoma. Cancer Genet Cytogenet 1990;46:143-156.

36. Das KM, Prasad I, Garla S, Amenta PS. Detection of a shared colon epithelial epitope on Barrett epithelium by a novel monoclonal antibody. Ann Int Med 1994;120:753-756.

37. Schmidt HG, Riddell RH, Walther B, Skinner DB, Riemann JF. Dysplasia in Barrett's esophagus. J Cancer Res Clin Oncol 1985;110:145-152.

38. Riddell RH. Grading of Dysplasia. Eur J Cancer 1995;31:1169-1170.

39. Blount PL, Galipeau PC, Sanchez CA, et al. 17p Allelic losses in diploid cells of patients with Barrett's esophagus who develop aneuploidy. Cancer Res 1994;54:2292-2295.

40. Benhattar J, Saraga E. Molecular genetics of dysplasia in ulcerative colitis. Eur J Cancer 1995;31:1171-1173.

41. Baker SJ, Preisinger AC, Jessup JM, et al. p53 Gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res 1990;50:7712-7722.

42. Huang Y, Boynton RF, Blount PL, et al. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. Cancer Res 1992;52:6525-6530.

43. Vogelstein B, Fearon ER, Hamilton SR. genetic alterations during colorectal tumor development. N Engl J Med 1988;319:525-532.

44. Sontag SJ, Schnell TG, Chejfec G et al. Barrett's oesophagus and colonic tumours. Lancet 1985;27:946-949.

SUMMARY

Chapter 1 is the general introduction of this thesis and gives an overview of the literature on Barrett's oesophagus, neoplastic progression in Barrett's oesophagus and oesophageal adenocarcinoma.

At the end of chapter 1 the aims of the study are presented. To determine if early diagnosis would benefit the patient, we analyzed the influence of tumour stage, differentiation grade and DNA-ploidy of tumour cells on the survival of patients with an adenocarcinoma in Barrett's oesophagus. Early diagnosis can be achieved by endoscopic surveillance of patients with Barrett's oesophagus. To create more efficient screening programs it is necessary to identify a subset of patients at high risk of developing carcinoma. To identify this subset we studied clinical characteristics (sex, age, extent of Barrett's mucosa, smoking and drinking habits) as well as histopathological factors (dysplasia, aneuploidy), which might indicate an increased risk of neoplastic progression. Finally we studied genetic changes by cytogenetic analysis to discover chromosomal aberrations common to all oesophageal adenocarcinomas, that may occur in early stages, so that these changes might provide markers for early cancer diagnosis.

Chapter 2.1 shows the results of a retrospective study of an 11 year period (1978-88) analyzing the survival of 112 patients with an adenocarcinoma in Barrett's oesophagus in respect of surgical treatment, tumour staging, and histological grading. Eighty-five patients (76%) underwent partial resection of the oesophagus and cardia. Postoperative mortality was 6%. After resection the 5-year survival was 24%. Survival was significantly better for patients without regional lymph node metastases (stage 0,I,IIA (n=61): 5-year survival 30%) and even better if the tumour was restricted to the submucosa (stage 0,I (n=12): 5-year survival 63%). There were no 5-year survivors after resection within the group of patients with regional lymph node metastases. Survival was not influenced by the histological grade of the tumour. Staging based on infiltration of the oesophageal wall and lymph node spread is valuable in determining the prognosis for patients with adenocarcinoma in Barrett's oesophagus.

Chapter 2.2 analyzes the role of DNA-ploidy of tumour cells as a prognostic

factor for the survival of patients with an adenocarcinoma in Barrett's oesophagus. Multivariate analysis showed a significant correlation between DNA-ploidy and survival after resection. Patients with diploid tumours had 3- and 5-year survival rates of 51% and 25% respectively compared to 10% and 0% respectively for patients with aneuploid or increased G2/tetraploid tumours. Although the number of patients in this study is relatively small, there is an indication that DNA-ploidy may be a prognostic factor for the survival of patients with an adenocarcinoma in Barrett's oesophagus.

Based on these findings, it can be concluded that early diagnosis will improve the prognosis of patients with an adenocarcinoma in Barrett's oesophagus.

Chapter 3 evaluates the importance of the length of columnar lined oesophagus, sex, age, smoking and drinking habits as risk factors for malignant degeneration. A case control study was performed comprising 96 patients with benign Barrett's oesophagus and 62 patients with adenocarcinoma in Barrett's oesophagus, diagnosed over the same period (1978-88). The extent of columnar lined oesophagus was related significantly to carcinoma: a doubling of the length resulted in a 1.7 times increased risk. Smokers had a 2.3-fold increased risk compared to nonsmokers. Male sex as risk factor approached statistical significance ($p=0.06$). Adjusted for these risk factors, no relation between carcinoma and age or alcohol consumption was found. The identification of these risk factors may help in developing more efficient screening programs for patients with Barrett's oesophagus.

Chapter 4 analyzes the role of dysplasia and aneuploidy as markers in columnar epithelium for malignant degeneration in Barrett's oesophagus. Dysplasia was found more often in columnar epithelium associated with adenocarcinoma compared with benign Barrett's oesophagus. Multivariate analysis using logistic regression showed an increased risk of malignancy in Barrett's oesophagus in case of dysplasia (odds ratio 9.4, $p=0.003$ for mild dysplasia and 33.1, $p<0.001$ for moderate or severe dysplasia). DNA-ploidy was not statistically significantly correlated with dysplasia. Aneuploidy or increased G2/tetraploidy proved to be an independent risk factor for younger patients (age <65 years: odds ratio 44.7, $p=0.003$). Dysplasia and aneuploidy or increased G2/tetraploidy in columnar epithelium are independent risk factors for malignant degeneration in Barrett's

oesophagus. Patients with either or both these risk factors should be offered at least annual or half yearly screening. Patients without these risk factors may be screened at greater intervals guided by other risk factors.

Chapter 5 shows the results of flowcytometric and cytogenetic analysis of Barrett's mucosa and adenocarcinoma of the distal oesophagus and cardia. Two of 8 analyzed specimens of Barrett's mucosa had clonal chromosomal abnormalities. Abnormal karyotypes with multiple and complex rearrangements were seen in 11 out of 37 adenocarcinomas, and single or a few numerical changes in 8. Losses of chromosomes 4, 18, 21 and Y were the most frequent numerical changes. Structural abnormalities were observed in 68% of the 19 abnormal karyotypes. The chromosome-arms most frequently rearranged were 1p, 3q, 11p and 22p. A chromosomal abnormality common to all tumours was not identified.

SAMENVATTING

Hoofdstuk 1 is de algemene inleiding van dit proefschrift en geeft een overzicht van de literatuur over de Barrett oesofagus, maligne ontaarding in Barrett slijmvlies en het adenocarcinoom van de oesofagus.

De doelstellingen van het onderzoek worden aan het einde van dit hoofdstuk geformuleerd. Om te bepalen of een vroegtijdige diagnose gunstig zou zijn voor de patient, werd de invloed van het tumor stadium, de differentiatiegraad en de DNA-ploidie van de tumorcellen op de overlevingsduur van patienten met een adenocarcinoom in een Barrett oesofagus bepaald. Vroegtijdige diagnostiek kan bevorderd worden door patienten met een Barrett oesofagus regelmatig endoscopisch te controleren. Om dit zo efficient mogelijk te doen is het van belang te bepalen welke patienten met name een verhoogde kans hebben op het ontstaan van een adenocarcinoom. Om deze patientengroep te kunnen definieren hebben we zowel klinische kenmerken (geslacht, leeftijd, uitbreiding van het Barrett slijmvlies, roken en alcoholgebruik) als histopathologische factoren (dysplasie, aneuploidie) bestudeerd. Tenslotte hebben we door middel van cytogenetisch onderzoek genetische afwijkingen bestudeerd in adenocarcinomen van de distale oesofagus en cardia, om mogelijke gemeenschappelijk aanwezige chromosomale afwijkingen op het spoor te komen, die al in een vroeg stadium zouden kunnen optreden en een vroege aanwijzing zouden kunnen zijn voor maligne ontaarding.

Hoofdstuk 2.1 beschrijft de resultaten van een retrospectief onderzoek over een periode van 11 jaar (1978-88) naar de overlevingsduur van 112 patienten met een adenocarcinoom in een Barrett oesofagus in relatie tot chirurgische behandeling, stadiering en histologische gradering van de tumor. Vijfentachtig patienten (76%) ondergingen een partiele resectie van de oesofagus en cardia. De postoperatieve sterfte bedroeg 6%. De 5-jaarsoverleving na resectie was 24%. De groep patienten zonder regionale lymfekliermetastasen had een statistisch significant betere overlevingsduur (stadium 0,I,IIA; 5-jaarsoverleving 30%) en de langste overlevingsduur werd gezien in de groep met tumor beperkt tot de submucosa zonder regionale lymfekliermetastasen (stadium O,I; 5-jaarsoverleving 63%). In de groep met regionale lymfeklier metastasen waren 5 jaar na

resectie geen patienten meer in leven. De overlevingsduur werd niet beinvloed door de histologische differentiatiegraad van de tumor. Stadiering gebaseerd op doorgroei van de tumor in de wand van de oesofagus en de aanwezigheid van regionale lymfekliermetastasen is zinvol voor het bepalen van de prognose voor patienten met een adenocarcinoom in een Barrett oesofagus.

Hoofdstuk 2.2 bestudeert de waarde van de DNA-ploidie van tumorcellen als prognostische factor voor de overlevingsduur van patienten met een adenocarcinoom in een Barrett oesofagus. Door middel van multivariate analyse werd een statistisch significante relatie aangetoond tussen DNA-ploidie en overlevingsduur na resectie. Patienten met diploide tumoren hadden een 3- en 5-jaars overleving van respectievelijk 51% en 25%, vergeleken met respectievelijk 10% en 0% voor patienten met aneuploidie of verhoogde G2/tetraploidie. Hoewel het aantal patienten in deze studie gering is, en er geen conclusie getrokken kan worden met betrekking tot de prognostische waarde, is er een aanwijzing dat DNA-ploidie een prognostische factor zou kunnen zijn voor de overlevingsduur van patienten met een adenocarcinoom in een Barrett oesofagus.

Gebaseerd op deze bevindingen kunnen we concluderen dat vroegtijdige diagnostiek de prognose van patienten met een adenocarcinoom in een Barrett oesofagus zal verbeteren.

Hoofdstuk 3 gaat in op de waarde van de uitbreiding van het Barrett slijmvlies, geslacht, roken en alcohol gebruik als risicofactoren voor maligne ontaarding. Door middel van een case control studie werden deze factoren vergeleken tussen een groep van 96 patienten met een Barrett oesofagus zonder maligniteit en een groep van 62 patienten met een adenocarcinoom in een Barrett oesophagus. Beide groepen werden gediagnostiseerd gedurende dezelfde periode (1978-88). Er was een statistisch significante relatie tussen de uitbreiding van het Barrett slijmvlies en maligniteit: verdubbeling van de lengte leidde tot een 1,7 maal verhoogd risico. Rokers hadden een 2,3 maal verhoogd risico vergeleken met niet-rokers. Mannelijk geslacht als risicofactor benaderde statistische significantie ($p=0,06$). Na correctie voor deze factoren werd geen relatie gevonden tussen leeftijd of alcohol gebruik en maligniteit. Rekening houdend met de aanwezigheid van deze risicofactoren zouden meer efficiente endoscopische controle schemata ontwikkeld kunnen worden voor patienten met een Barrett oesopfagus.

In **hoofdstuk 4** wordt de rol van dysplasie en aneuploidie als indicatoren voor maligne ontaarding in een Barrett oesofagus geanalyseerd. Dysplasie werd vaker aangetroffen in Barrett epitheel indien een carcinoom aanwezig was dan in Barrett epitheel zonder carcinoom. Door middel van multivariate analyse werd aangetoond dat er een verhoogde kans bestaat op maligniteit in een Barrett oesofagus als er dysplasie van het epitheel aanwezig is (odds ratio 9,4, $p=0,003$ voor geringe dysplasie en odds ratio 33,1, $p<0,001$ voor matige en ernstige dysplasie). Dysplasie en aneuploidie of verhoogde G2/tetraploidie in Barrett epitheel zijn onafhankelijke risicofactoren voor maligne ontaarding. Patienten met deze risicofactoren zouden tenminste eens of tweemaal per jaar endoscopisch gecontroleerd moeten worden. Patienten zonder deze risicofactoren hoeven minder vaak gecontroleerd te worden, afhankelijk van de aanwezigheid van andere factoren.

Hoofdstuk 5 geeft de resultaten weer van flowcytometrie en cytogenetisch onderzoek van Barrett epitheel en adenocarcinoom van de distale oesofagus en cardia. In 2 van de 8 geanalyseerde weefselstukjes met Barrett epitheel werden clonale chromosomale afwijkingen gevonden. Afwijkende karyogrammen met multipele en complexe afwijkingen werden in 11 van de 37 adenocarcinomen gevonden, en een of meerdere numerieke afwijkingen in 8. De meestvoorkomende numerieke afwijkingen waren verlies van chromosoom 4,18,21 en Y. Structurele afwijkingen waren aanwezig in 68% van de 19 adenocarcinomen met afwijkende karyogrammen. Structurele afwijkingen werden vooral gezien op chromosoomarm 1p, 3q, 11p en 22p. Een specifieke chromosomale afwijking voor alle tumoren werd niet gevonden.

DANKWOORD

Dit proefschrift is tot stand gekomen dankzij de medewerking, adviezen en het enthousiasme van veel mensen. Op deze wijze wil ik iedereen bedanken die op enigerlei wijze een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift. Met name wil ik dank zeggen aan:

Mijn promotor, Prof. Jeekel, voor de mogelijkheid om met wetenschappelijk onderzoek te starten en de vrijheid die ik gekregen heb bij het verrichten daarvan.

Mijn co-promotor, Dr. Tilanus, voor zijn enthousiaste ondersteuning en begeleiding van het onderzoek vanaf het eerste moment.

Prof. Hagemeyer, voor de mogelijkheid om cytogenetisch onderzoek te verrichten, de enorme hoeveelheid energie die zij daar in gestoken heeft en de kritische beoordeling van het manuscript.

De leden van de promotiecommissie, Prof. van der Kwast en Prof. Wilson voor de beoordeling van het manuscript.

Dr. van Blankenstein voor de ideeënstroom voor onderzoek en de bereidheid om plaats te nemen in de grote commissie.

Prof. Obertop en Prof. Stockbrügger, voor hun bereidheid om plaats te nemen in de grote commissie.

Ir. Hop voor de statistische ondersteuning van de verschillende onderzoeken.

Dr. Mulder, voor de introductie in de flowcytometrie, het beoordelen van een grote hoeveelheid coupes en het meten van vele monsters op de FACS.

Dr. Dees voor de medewerking bij het verzamelen van patientgegevens en materiaal.

Ellen, Hanneke, Bep en alle medewerksters van het laboratorium voor Klinische Genetica voor hun inzet, enthousiasme en plezierige samenwerking bij het onderzoek.

Kees Vissers voor de hulp bij het voorbereiden van de weefsels voor flowcytometrie.

De medewerkers van de operatiekamers, GE-centrale en PA-afdeling voor de hulp bij het verzamelen van materiaal.

Laura van Dortmont en Jeannette Schoonderbeek voor hun steun als paranimf.

Roland, voor het vormgeven van de gecompliceerde tabellen in hoofdstuk 5 en voor alle steun waardoor het mogelijk was om dit proefschrift te schrijven.

CURRICULUM VITAE

5 februari 1964	Geboren te Enschede
1976 - 1982	Gymnasium-B, Jacobus College, Enschede
1982 - 1989	Studie Geneeskunde, Erasmus Universiteit Rotterdam
1989 - 1991	Wetenschappelijk onderzoeker, afdeling Heelkunde Academisch Ziekenhuis Rotterdam - Dijkzigt (Begeleiders: Prof. Dr. J. Jeekel, Dr. H.W. Tilanus)
1991 - 1992	Assistent geneeskundige niet in opleiding, afdeling Heelkunde Academisch Ziekenhuis Rotterdam - Dijkzigt (Hoofd: Prof. Dr. J. Jeekel)
1992 - 1995	Assistent geneeskundige in opleiding, afdeling Heelkunde Reinier de Graaf Gasthuis, Delft Opleider: Dr. W.B.J. Jansen
1995 - heden	Assistent geneeskundige in opleiding, afdeling Heelkunde Academisch Ziekenhuis Rotterdam - Dijkzigt Opleider: Prof. Dr. H.A. Bruining Hoofd: Prof. Dr. J. Jeekel