SOME OBSERVATIONS ON THE EPIDEMIOLOGY OF
BARRETT’S OESOPHAGUS AND ADENOCARCINOMA OF
THE OESOPHAGUS

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SOME OBSERVATIONS ON THE EPIDEMIOLOGY OF BARRETT’S OESOPHAGUS AND ADENOCARCINOMA OF THE OESOPHAGUS

Enige waarnemingen omtrend de epidemiologie van de Barrett oesophagus en het adenocarcinoom van de oesophagus

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Preface

The rapidly increasing incidence of adenocarcinoma of the oesophagus, a cancer which arises in Barrett’s oesophagus, has created a flurry of interest in this pre-malignant condition. The cancer risk involved in Barrett’s oesophagus was variously reported to be between 0.23 and 2% per annum and especially the higher estimates resulted in attempts to control the incidence of adenocarcinoma of the oesophagus by endoscopic surveillance. However, this proved to be of limited use as only 5% of individuals with adenocarcinoma of the oesophagus were found to have been previously identified with Barrett’s oesophagus.

The gastroenterological community in the old Rotterdam University Hospital had a longstanding interest in Barrett’s oesophagus and its associated adenocarcinoma. Jan Dees described one of the first large series of patients with adenocarcinoma in Barrett’s oesophagus. He also was able to conduct a number of the most extensive observational studies into the incidence of adenocarcinoma in Barrett’s oesophagus, which found adenocarcinoma of the oesophagus incidence rates in the 0.5% per annum range and, in addition, demonstrated that in practice, only a small the percentage of patients with Barrett’s oesophagus actually died from adenocarcinoma of the oesophagus. In addition, a number of clinical and basic studies from the departments of Surgery and Pathology and lately from the department of Gastroenterology and Hepatology have maintained Rotterdam in the forefront of Barrett’s oesophagus research.

After my retirement a survey of the literature on the epidemiology of Barrett’s oesophagus produced surprisingly few items. However, there were a number of publications containing data which appeared to offer opportunities for further analysis by an author with more leisure time than the original authors. These authors who very generously shared their data with me were Dr. Clarisse Böhmer (Haarlem, The Netherlands), Dr. Peter Bytzer (Copenhagen, Denmark) and Dr. Christine Caygill (London, UK) with whom I was able to co-author the first 4 publications in this thesis.

These publications set me on the path of the study of the epidemiology of cancer of the oesophagus in The Netherlands on the basis of the extensive data provided by the Netherlands Cancer Registry in Utrecht. Finally, the study testing the hypothesis of an inverse relationship between the incidence of adenocarcinoma of the oesophagus and the prevalence of H. pylori infection (MDL laboratory, Erasmus MC Rotterdam), added a small element of do it myself.

Mark van Blankenstein

Berkel en Rodenrijs, November 2006
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Chapter 1

GENERAL INTRODUCTION AND AIM OF THE THESIS
AIM OF THIS THESIS.

The fact that the presence of Barrett’s oesophagus is thought to be unknown in at least 90% of individuals\(^1\) suffering from this pre-malignant condition\(^2\) has obviously been the central handicap in describing its epidemiology. The well known analogy with an iceberg is very apt. It is in principle possible to obtain data on the height, circumference and angle of slope of the iceberg from observations of its visible part. Although the number of observers of the visible Barrett iceberg has been small, because each viewed different areas, they have tended to come up with a variety of answers. The submerged edges of the iceberg regularly collide with other objects floating in its vicinity; we then speak of adenocarcinoma of the oesophagus. These collisions obviously reveal the location of some of the outer edges of the iceberg and should be able to provide data from which it may be possible to construct a description of its hidden 90%.

This thesis comprises a number of studies attempting to gain more insight into the epidemiology of Barrett’s oesophagus, on the one hand from observing the visible part of the iceberg from the broadest possible viewpoint, i.e. relatively unselected patients referred for endoscopy by general practitioners or randomly selected individuals who are part of an isolated population, on the other hand, from the palpable results of the collisions, the epidemiology of adenocarcinoma of the oesophagus and its companion, adenocarcinoma of the gastric cardia.

Outline of the thesis.

**Chapter 1,** a general introduction. After defining and describing Barrett’s oesophagus, it then traces the history of its development and its relationship to both its predecessor, reflux oesophagitis and to its outcome, adenocarcinoma of the oesophagus. Next an outlines is given of the history of reflux oesophagitis. Finally, it provides a brief overview of current thinking about carcinogenesis in Barrett’s oesophagus and its relationship to gastro-oesophageal reflux.

**Chapter 2** gives an analysis of the incidence of adenocarcinoma in inhabitants of institutions for the severely mentally handicapped, in whom the prevalence of Barrett’s oesophagus had previously been determined in a randomly selected, but representative group. This population was unique, both in its lack of mobility, its social homogeneity and in not consuming alcohol or tobacco.

**Chapter 3** describes the prevalence of Barrett’s oesophagus in a population of patients referred over a 15-year period by general practitioners for endoscopy to a single endoscopic unit in the UK. This relatively unselected patient population hopefully provided the closest possible approach to an unselected cross section of the general population. By using the total number of endoscopies as the denominator, it was possible to establish Barrett’s oesophagus prevalence rates by age and gender.

**Chapter 4** describes the calculation of the age and gender specific incidence rates of adenocarcinoma of the oesophagus, adenocarcinoma of the gastric cardia and squamous cell carcinoma of the oesophagus in Denmark. This material was unique as the always difficult distinction between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia had been made by a panel of gastroenterologists, using original diagnostic data.
Chapter 5 reports the first attempt to deduce the size and composition of the invisible Danish population with Barrett’s oesophagus from the incidence data for adenocarcinoma of the oesophagus established in chapter 4 by the use of a little known statistical method named expectation maximalisation.

Chapter 6 provides an analysis of the time trends in the incidence rates of adenocarcinoma of the oesophagus and of adenocarcinoma of the gastric cardia in The Netherlands over the period 1989-2003. It aimed to establish whether both tumours had been equally involved in the overall rise in the incidence of oesophageal adenocarcinomas in The Netherlands over this 15 year period.

Chapter 7 comprises the first population based study attempting to explain the considerable differences in the incidence rates of adenocarcinoma of the oesophagus in 9 Comprehensive Cancer Registration regions of The Netherlands from differences in the prevalence of *Helicobacter pylori* infection in four of these regions with contrasting incidence rates. The prevalence of *Helicobacter pylori* infection was determined in 400 blood donors from each of these four regions, divided into 5 ten-year age groups.

Chapter 8 the final chapter, contains a summary and a discussion of the findings presented in this thesis.
Barrett’s oesophagus

Definition

Barrett’s oesophagus, more scientifically known as columnar lined oesophagus, is the
eponymous description of the metaplastic change from squamous to columnar epithelium
occurring in the lining of the distal oesophagus.
Barrett’s oesophagus can extend over distances ranging between a few millimetres to less than 3
cm in short or ultra-short segment Barrett’s oesophagus or to more than 3 cm in long segment
Barrett’s oesophagus. As is the case with other metaplastic epithelia, Barrett’s oesophagus is a
pre-malignant condition which precedes practically all adenocarcinomas of the oesophagus.
Originally, three main histological types of Barrett’s oesophagus were described. These were
atrophic gastric fundic-type epithelium with parietal and chief cells; junctional-type epithelium
with cardiac mucous glands and intestinal-type epithelium with a villiform surface, mucous
glands and intestinal-type goblet cells. A notable finding was that, when present, specialized
columnar epithelium was almost always the most proximal and gastric fundic epithelium the
most distal, with junctional epithelium interposed between these two. Such zonation has since
both been confirmed and denied in series of oesophageal resection specimens containing
adenocarcinoma of the oesophagus in Barrett’s oesophagus.
The finding that only specialised intestinal metaplastic epithelium was associated with
adenocarcinoma of the oesophagus, resulted in the definition of Barrett’s oesophagus being
narrowed down to those cases where specialised intestinal metaplastic epithelium was present.
Although this refinement had pragmatic advantages in limiting the number of patients
qualifying for endoscopic surveillance, it obviously raised the question what, if any, was the
prognostic relevance of columnar lined oesophagus without specialised intestinal metaplastic
epithelium.
Surprisingly little, or rather, no attention has been focussed on the further natural history of
columnar lined oesophagus without specialised intestinal metaplastic epithelium. The present
definition would appear to imply the existence two separate forms of metaplasia, one pre-
malignant and the other innocent. However, in resected cases of adenocarcinoma of the
oesophagus, both specialised intestinal metaplastic epithelium and gastric mucosa have been
shown to exist together in the same specimen.
In a large series of long segment Barrett’s oesophagus patients, the prevalence of specialised
intestinal metaplastic epithelium ranged from 71% in Barrett’s oesophagus segments of 3-6 cm
to 81% in 6.1-10 cm segments and 100% in segments > 10.1 cm. In addition, cardiac mucosa
was also found in all cases, again confirming that both types of metaplasia co-exist and may
represent either parallel or consecutive stages of metaplasia.
Clinically, the UK National Barrett’s Oesophagus Registry follow up of 232 patients with long
segment Barrett’s oesophagus over 402 patient years, found a 0.5% adenocarcinoma of the
oesophagus incidence rate for both patients with and without specialised intestinal
metaplastic epithelium at their initial biopsy. Obviously, in a number of cases the absence of
specialised intestinal metaplastic epithelium may well have been the result of sampling error;
however, this study calls into question the validity of limiting the diagnosis of Barrett’s
oesophagus for prognostic purposes to the presence of specialised intestinal metaplastic
epithelium. It is therefore understandable that the British Society of Gastroenterology has
recently abandoned the requirement of specialised intestinal metaplastic epithelium for the
diagnosis of Barrett’s oesophagus\textsuperscript{17}.

\textbf{Anatomical boundaries}

The proximal boundaries of Barrett’s oesophagus are clearly delineated and their recognition
only requires attention to detail from the endoscopist. Although it is not unusual to find some
erosive oesophagitis above the proximal limit of Barrett’s oesophagus, this upper limit would in
general appear to be stable\textsuperscript{18}, although in the older literature, before effective treatment was
available, the upward ascent of the squamo-columnar junction was occasionally observed\textsuperscript{19-23}.
The distal boundary presents a far more complicated problem. In principle, Barrett’s oesophagus
comprises all columnar epithelium proximal to the original squamo-columnar junction\textsuperscript{24}.
However, in view of the similarity between the columnar epithelium of the cardia and columnar
lined oesophagus, in Barrett’s oesophagus this landmark is no longer recognisable. Columnar
epithelium found at a distance greater than 2 cm above the proximal margin of the gastric folds
currently offers a popular surrogate marker for the original squamo-columnar mucosal junction\textsuperscript{25}.
However, for careful endoscopists, a remnant of the palisadal capillary pattern above the original
squamo-columnar junction is frequently still visible\textsuperscript{26} and can often best be seen in high
retrovision (personal observation).
HISTORICAL OVERVIEW

Barrett’s Oesophagus

In 1950 Barrett published his paper “Chronic peptic ulcer of the oesophagus and oesophagitis” with which he hoped to resolve the existing interdisciplinary confusion between these two entities. He argued, citing Allison, that reflux oesophagitis caused superficial ulceration, fibrosis and shortening of the oesophagus producing an intrathoracic segment of stomach in which peptic gastric ulcers can develop, a condition previously described by Allison, Johnstone and Royce. Barrett was, in fact, at pains to deny the existence of the condition which subsequently bore his name. He did however, subsequently change his mind in 1957 in a paper titled: “The lower esophagus lined by columnar epithelium.” His most novel observation was that “a benign stricture at the level of the aortic arch should suggest that the lower esophagus is lined by columnar epithelium because sliding hiatal hernias are not usually as large as this.” He considered the columnar lined oesophagus was “probably the result of a failure of the embryonic lining of the gullet to achieve normal maturity”.

In 1951 Bosher and Taylor described a case with a peptic stricture in the oesophagus at the level of the aortic arch and the distal oesophagus lined by gastric mucosa. Two years later, Allison and Johnstone described 7 similar patients in whom, on the basis of anatomical landmarks i.e. the absence of peritoneal covering and the presence of squamous islands, they concluded the oesophagus to be lined by a gastric type of mucosa. These patients also had sliding hiatal hernias, a condition which Allison had previously described in combination with gastro-oesophageal reflux, and superficial peptic ulceration and stricture of the oesophagus. They mentioned the possibility that the healing of ulcerated squamous epithelium in an acid environment might involve the overgrowth by gastric epithelium.

In 1961 this hypothesis was expanded by Hayward in a rather iconoclastic publication in which he criticised his predecessors. He ridiculed the concept of a congenital cause for gastric epithelium in the oesophagus, citing a series of 25 cases with an average age 62 in whom columnar epithelium had been found to extend an unusual distance up the oesophagus. He summarised the current views as follows: “Medical reasoning about an abnormally long length of oesophagus lined by junctional epithelium seems to have proceeded along the following lines: 1) The oesophagus is lined by squamous epithelium. 2) Oesophagus lined by columnar epithelium is not oesophagus. 3) Since columnar epithelium is not oesophageal, it must be gastric. 4) Therefore columnar epithelium in the oesophagus must be ectopic gastric epithelium. 5) As it is ectopic, it must be congenital. It would not matter if this reasoning remained on the theoretical plane without practical consequence, but this is not the case. There are two more steps in the reasoning. 6) Congenital anomalies are permanent. 7) There is only one thing to do with parts which are permanently abnormal, are causing trouble, and can be done without, and that is to cut them out.” He defined the oesophago-gastric junction as a segment of junctional mucosa within which he projected the cardia as the functioning muscular sphincter. He then argued that, reflux from the stomach, resulting from a failure of the cardia and caused by a sliding hiatal hernia, digested the squamous epithelium. This allowed metaplastic junctional epithelium to creep up to the level of the aortic arch or higher. He believed this process to be reversible. A masterful overview was given by Adler in 1963 in which he considered the two possible causes of columnar lined oesophagus. He rejected the congenital theory, according to which columnar lined oesophagus resulted from an incomplete replacement of the columnar epithelium.
by squamous epithelium during foetal development. As this replacement starts in mid-oesophagus and progresses to each end, there was no reason for columnar lined oesophagus only to be found in the distal oesophagus. On the basis of the observed ascent of the squamo-columnar junction observed by a number of authors¹⁹-²², he opted for the acquired theory in which columnar lined oesophagus resulted from “an adaptive epithelial change in response to the chronic abnormal (acid-pepsin) environment of the oesophagus”. In addition, if the columnar lined oesophagus was congenital in origin and produced acid, it would produce symptoms at an early age instead of the observed incidence in middle or late life. He summarised three possible sources of the columnar lined oesophagus: extension upward from the stomach, metaplasia and extension from the cardiac glands, finally opting for the latter as the glands were seen to hypertrophy, possibly to protect the deeper layers of the oesophagus. He also advanced the possibility that adenocarcinomas originated in columnar lined oesophagus, citing Morson³⁷ who had suggested that repeated attacks of inflammatory gastritis led to a pre-malignant intestinal metaplasia similar to that encountered in the stomach.

In 1966 Mossberg reported a young man with “psychogenic vomiting, in whom 3 oesophagoscopic biopsies taken over a 32 months period, confirmed the appearance of columnar epithelium in areas which had previously contained normal squamous epithelium. He concluded that the “ascent” of the columnar lining resulted “from gastro-oesophageal reflux, oesophagitis and regeneration of the destroyed oesophageal lining by columnar rather than squamous epithelium”²³.

In 1970 Bremner et al. were the first to succeed in inducing columnar lined oesophagus experimentally in a canine model. They dissected 6-10 cm of distal oesophageal mucosa away from the muscular wall in 3 groups of dogs. In 2 groups, reflux was induced by creating a hiatal hernia and performing cardioplasty. In addition, acid production was stimulated by injections of histamine in 1 of the 2 reflux groups. The most extensive columnar regeneration was seen in dogs with reflux and histamine stimulation, the least in dogs without reflux. This study demonstrated that, under conditions of gastro-oesophageal reflux, destroyed squamous epithelium was replaced by columnar epithelium and that the degree of acidity of the refluxate influenced the extent of this metaplasia. In common with Hayward,³⁵ the source of the columnar lined oesophagus was thought to be upward migration of gastric or junctional epithelium³⁸.

The latter hypothesis was challenged by Gillen et al. in 1988. In their canine model a 2-cm high circumferential ring of squamous mucosa was removed from the distal mucosa, leaving a small strip intact to identify the squamocolumnar junction. Above this ring a 2-cm high ring was left intact and above this, a further 2-cm high ring was resected. Four groups of dogs were, in addition, subjected to: 1) cardioplasty, hiatal hernia and pentagastrin injections³⁸, 2) cardioplasty, hiatal hernia and a common bile duct ligation with cholecystogastrostomy (bile+ acid group), 3) similar to group 2, with additional cimetidine treatment, 4) only the two circumferential resections, the lower oesophageal sphincter left intact. A fifth group, unoperated before sacrifice, supplied multiple longitudinal sections from lower oesophagus to upper stomach as normal histological controls. Columnar lined oesophagus was only found in both rings in 2/6 dogs in group 1. In group 2, columnar lined oesophagus was only found in the lower ring of 3/6 dogs while in group 3, under cimetidine, no columnar lined oesophagus was found. Where columnar lined oesophagus was absent, regeneration was by squamous epithelium. This study showed that it was possible for columnar lined oesophagus to arise in areas not contiguous with gastric or junctional epithelium. There was no additive effect of bile while acid inhibition prevented regeneration by columnar lined oesophagus. In ulcerating areas, where re-
epitheliasation was ongoing, this was seen to be extending from the necks of oesophageal gland ducts, suggesting that these were the source of the columnar epithelium. This concept was in turn challenged by a study by Seto and Kobori in a rat model in which total gastrectomy and oesophago-jejunal anastomosis induced bile and pancreatic reflux. In addition, half of the rats drank syrup with HCl at a pH of 1.8. Reflux oesophagitis was found in all rats, in those sacrificed at 12 weeks all 3 had developed columnar lined oesophagus. In some, islands of columnar epithelium surrounded by squamous epithelium and distant from the site of the anastomosis were found. As rats do not possess oesophageal glands, these islands must have resulted from metaplasia of the squamous epithelium.

These studies, having lain to rest the congenital origin of Barrett’s oesophagus and having confirmed the link to gastro-oesophageal reflux disease, resulted in the search for the aetiology of Barrett’s oesophagus shifting to the causes of reflux oesophagitis.

**Barrett’s oesophagus and adenocarcinoma of the oesophagus**

In 1950 Carrie described a case of “adenocarcinoma of the upper end of the oesophagus arising from ectopic gastric epithelium.” , currently known as the inlet patch. He cited Hewlett who suspected that this ectopic gastric mucosa could give rise to adenocarcinoma but found that none of the 6 published cases had occurred in the upper oesophagus. He consequently remarked: “Since then, adenocarcinoma of the upper end of the oesophagus had become somewhat like a unicorn, for while the authors of most text-books state that this tumour does occur, they do not state that they have seen it themselves, and fail to state who did.” None of the 19 cases of adenocarcinoma of the oesophagus seen in his department over the previous 15 years involved this area.

The first clear case of adenocarcinoma of the oesophagus in Barrett’s oesophagus was published by Morson and Belcher in 1952. The patient, a male aged 56, underwent a partial oesophageal resection for an adenocarcinoma at the level of the tracheal bifurcation. The mucous membrane above the tumour was squamous, below glandular, apart from a few islands of squamous epithelium. The distal part of the resected specimen “corresponded closely to normal cardiac type gastric mucous membrane”. “However, most of it showed chronic inflammatory and atrophic change with a tendency towards an intestinal type containing many goblet cells.” The authors commented on the rarity of adenocarcinoma of the oesophagus but considered it to be an entity which could arise in islands of ectopic gastric mucosa, or, as in this case, from a congenital abnormality of the oesophagus lined by a gastric type of mucosa. Morson’s description, as was to be expected from this outstanding pathologist, encompassed all the features of what is now known as adenocarcinoma of the oesophagus in Barrett’s oesophagus, including the finding that the histological changes increased in an oral direction. There may, however, have been an earlier case in the literature. In a 1935 case record from the Massachusetts General Hospital, a 61-year old male was presented suffering from coronary artery disease who developed upper abdominal discomfort and mild dysphagia. A x-ray examination (by Richard Schatzki who had by then emigrated from Germany to the US) revealed an oesophageal diverticulum at the level of the aortic arch, where there were also changes suggestive of varices and a distal stricture over 4 cm with the fundus of the stomach protruding through the diaphragmatic hiatus. An oesophagoscopy was stated to have found haemorrhagic mucosa at 11.5 inches, i.e. 29 cm. from the front teeth. The biopsy was reported as epidermoid carcinoma. After a symptomatic remission on x-ray therapy the patient died and autopsy was performed. The tumour proved to be
an adenocarcinoma extending upwards from the ‘cardiac orifice’. The “varices” were found to have been submucosal metastases, extending along the lymphatics and mostly covered by normal squamous epithelium. Mallory, the pathologist provided a beautiful description of this now classic endoscopic finding which is only seen with adenocarcinomas, but which was then for him a novelty\textsuperscript{43}. Was this a true case of adenocarcinoma of the oesophagus in Barrett’s oesophagus? It was obviously an adenocarcinoma of the gastro-oesophageal junction, there is no mention of tumour in the fundus and there was a hiatal hernia which was, of course, spotted by Schatzki. Another early case of adenocarcinoma of the oesophagus in Barrett’s oesophagus was mentioned, but not commented on, by Allison and Johnstone in their 1953 paper\textsuperscript{33}. A third case was described by Thomas et al in 1954, who apparently were not aware of the Morson case. They did cite 6 cases described by Hewlett in 1900\textsuperscript{42} and one by Feldman in 1939\textsuperscript{44}.

In 1956 Smithers reviewed a number of cases of adenocarcinoma of the oesophagus. His case series, which was started in 1936, while illustrating some of the confusion still attending this subject, was also one of the first to distinguish the adenocarcinomas of the oesophagus, the gastric cardia and the gastro-oesophageal junction. He cited 6 ways in which adenocarcinoma of the oesophagus were thought to arise, 1. from ectopic islets of gastric mucosa, 2. a section of the oesophageal mucosa which had failed to undergo squamous transformation before birth, 3. mucosa which had undergone some glandular metaplasia following attrition or long standing infection, 4. minor extensions or folds of gastric mucosa lining the hiatal canal, 5. a congenital short oesophagus and 6. lymphatic spread of gastric tumours. It is interesting to note that this at first sight strange collection was actually describing Barrett’s oesophagus in items 2, 3 and 5 and short segment Barrett in 4. Smithers noted that if adenocarcinomas did arise in superficial glands they should also be seen at the proximal end of the oesophagus surrounded by squamous epithelium, the earlier cited unicorn. He also pointed to the difficulty in distinguishing between adenocarcinoma of the gastric cardia and the oesophagus. He finally concluded that the oesophageal mucosa was not uncommonly partly lined by gastric mucosa and the interest in hiatus hernia had resulted in the more frequent diagnosis of oesophageal adenocarcinoma and the acceptance of the existence of this tumour. In spite of the fact that he considered that oesophageal adenocarcinoma mainly arose in mucosa which has failed to undergo squamous transformation, he continued to divide his cases of adenocarcinoma of the oesophagus into 26 without and eight with an hiatal hernia\textsuperscript{45}.

In 1968 Lortat-Jacob described 16 cases of primary oesophageal adenocarcinoma found in the resected specimens of 558 patients undergoing resection for oesophageal cancer. He excluded 85 cases which he considered to have originated in the cardia and cases originating from gastric cancer metastases. He distinguished three groups, the first in which the tumour developed above the cardia and was surrounded by squamous epithelium. In one case the tumour was located at 32 cm with squamous epithelium distal to it. In the other two cases the anatomical relationship with the cardia was not described. The second group comprised 7 cases of “squamous and glandular carcinomas and cylindromatous carcinomas” localised in the middle and distal third of the oesophagus and finally 6 cases of adenocarcinoma in columnar epithelium. The latter he considered to be caused by malignant degeneration of peptic ulcers of the “endo-brachy-oesophagus as Barrett’s oesophagus was termed in France. He considered that these tumours “must be distinguished from adenocarcinomas of the cardia associated with a short oesophagus”, a difficult distinction which suggests that some of the tumours classified as adenocarcinomas of the cardia were in fact adenocarcinoma of the oesophagus\textsuperscript{46}.
The publication which put adenocarcinoma of the oesophagus squarely on the map was by Naef et al. who found 12 cases of adenocarcinoma of the oesophagus in 140 patients with extensive Barrett’s oesophagus. These had been identified in 3,981 patients with hiatal hernia and reflux, in 1,225 of whom reflux oesophagitis had been found. In a number of patients the upper level of Barrett’s oesophagus had been seen to ascend at repeated endoscopies, thus finally proving the acquired nature of Barrett’s oesophagus. He considered that the 8.5% incidence of adenocarcinoma of the oesophagus in Barrett’s oesophagus to indicate a significant causal relationship. In 1978 a series of 71 oesophageal cancers referred for surgery to Rotterdam over a 4-year period and seen in the endoscopy unit of the Rotterdam University Hospital, was reported to the British Society of Gastroenterology. Forty-four were squamous cell cancers and 27 adenocarcinomas. Of the latter, 16 were adenocarcinoma of the oesophagus in Barrett’s oesophagus, none of which had previously been recognised as such.

Having identified this premalignant condition, the medical profession was confronted with the question of: what to do? The obvious answer was prevention, treatment or if both were impossible, endoscopic surveillance. In 1983 Spechler voiced a certain enthusiasm for this approach. However, a year later, after observing an incidence of only one adenocarcinoma in 175 patient-years, Spechler already questioned the value of endoscopic surveillance for patients with Barrett’s oesophagus. However, this view proved unpopular in a dynamic profession and a spate of publications appeared over the next 15 years, reporting far higher cancer incidences than found by Spechler and emphasising the importance of endoscopic surveillance. A considerable number with the highest cancer incidence rates probably owed their appearance in print to publication bias.

In Rotterdam, where annually scores of patients with adenocarcinomas in Barrett’s oesophagus were seen and consequently the clinical importance certainly not underestimated, Dees decided to approach the problem from the viewpoint of the risk run by patient with Barrett’s oesophagus. He organised a follow up of 155 patients with Barrett’s oesophagus, diagnosed between 1973 and 1986. In two consecutive observational studies, separated by 8 years, he found a constant adenocarcinoma incidence of 1/180 patient-years. In addition he established that of the 79 patients who had died, 5 had developed adenocarcinoma of the oesophagus but only 2 had died from their tumour. At a third review in 2002, the incidence rate was still found to be the same. In addition, it revealed that 44 survivors, diagnosed at an average age of 49.6 years, had experienced an average ACO free interval of 20 years (range 16.3-26.5 years). The debate remains ongoing.
REFLUX OESOPHAGITIS.

Origins of the concept

The description of this condition originated from two different disciplines, radiology and endoscopy. In 1925 Friedenwald and Feldman described the radiological and clinical findings of what would now be termed a hiatal hernia. This concept was developed by von Bergmann in Germany and by Hurst in Britain. Hurst, citing Knothe and Schatzki, noted that the radiological findings could frequently be induced in asymptomatic individuals by increasing intra-abdominal pressure, especially in patients over the age of 60. However, there were a number of patients with symptoms of: “pain or a feeling of pressure immediately after swallowing, under the xiphisternum or a little to the left, and occasionally in the back; it may radiate to the heart and to the left arm and may closely simulate angina. Acid regurgitation is common; occasionally vomiting is the only symptom.” “The attacks are in most cases mainly or exclusively nocturnal and disappear on sitting up; severe night pain may simulate gall-stones. Intermittent dysphagia may occur.” To prevent nocturnal attacks, raising the head of the bed as far as possible was advised, an approach which was still popular until the introduction of effective acid suppression in the 1980’s. However, the link between, gastro-oesophageal reflux oesophagitis and the radiological diagnosis of hiatal hernia had not yet been laid.

According to Cross, reflux oesophagitis was first described by Quinke in 1879 and by MacKenzie in 1884. However, the present endoscopic concept of peptic oesophagitis was formulated by Winkelstein in 1935 on the basis of five cases. He described it as a chronic disease of elderly men “characterized by exacerbations and remissions resembling those of peptic ulcer.” Three patients had duodenal ulcer, one a peptic ulcer of the oesophagus and one developed a lesser curve ulcer. All had “hyperchlorhydria” on the basis of then current tests. In addition, “the types of substernal pain, heartburn, sour regurgitations and the hyperchlorhydria in all (patients vB.) recall the clinical features of peptic ulcer of the esophagus which have been described in this country by Jackson and Friedenwald.” “However, the esophagoscopy (and radiography, which had shown spasm and irregular narrowing (and was the usual indication for endoscopy, vB.) in the cases described here reveals a diffuse inflammation without a definite ulcer”. He concluded that this “peptic esophagitis” probably resulted “from the irritant action on the mucosa of free hydrochloric acid and pepsin”. Chevalier Jackson, a prominent oesophagoscopist of that era, was in “hearty accord” with Winkelstein and pointed out the relation of this condition with the ‘herniated stomach’ and that “the chief reason..... why so little has been heard of peptic esophagitis is that so few esophagogoscopies are done in patients with gastric symptoms”. This lack of (rigid) endoscopic examinations and the fact that peptic ulcer of the distal oesophagus could simply be diagnosed by radiology, resulted in it remaining the most commonly diagnosed form of peptic oesophageal disease during the nineteen-forties and -fifties.
MECHANISMS OF REFLUX OESOPHAGITIS.

Anatomical studies.

In his 1946 paper Allison stated that “ulcer (of the oesophagus vB.) occurs where there is such a derangement of the mechanism of the cardia that acid gastric juice flows back easily into the lower end of the esophagus”. “The disorder which predisposes to ulceration is hernia of the stomach through the diaphragmatic hiatus into the posterior mediastinum.” He operated on two patients with a radiological diagnosis of short oesophagus and found that “the stomach could be replaced easily in its normal position in the abdomen without tension in the esophagus.” This may have undermined his belief in the short oesophagus. In his 1948 paper he summarised the anti-reflux mechanisms of the cardia as the combination of the angle between the oesophagus and the fundus and the thick walled tunnel of the diaphragmatic crura, which, by contracting during inspiration, prevent both the stomach contents from passing up the oesophagus and the cardia from being drawn into the mediastinum by the increased pressure within the abdomen and the suction from the chest. This mechanism was obviously lost in sliding hiatal hernias of the cardia and by the presence of heterotopic gastric mucosa in the oesophagus. He noted that in para-oesophageal hiatal hernias where the cardia remained below the diaphragm, the cardia remained competent. In 1951 Allison described 206 cases of hiatal hernia, 170 of which were sliding hernias, 34 more or less para-oesophageal and only one a congenital short oesophagus. He also described the application of metal Cushing brain clips to the squamocolumnar junction to observe its localisation radiographically, a technique which was recently revived. He now emphasised the role of the right crus of the diaphragm which, after splitting to form the hiatus, formed a sling for the oesophagogastric junction, and in analogy with the pubo-rectalis sling, compressed it against the spine. In addition, it maintained the angle of His. In 1959 Cross et al found hiatal hernias in 103/ 130 patients with oesophagitis. However, the importance of such anatomical aspects, and specifically the relevance of the crural diaphragm and consequentially, hiatal hernia, on oesophagitis, was to undergo considerable ups and downs over the following decades as the focus of attention shifted to the lower oesophageal sphincter. A study by Cohen and Harris published in 1971 and titled “does hiatus hernia affect competence of the gastrooesophageal sphincter” signalled the nadir of the hiatus hernia. These investigators compared patients with severe symptoms of gastroesophageal reflux and asymptomatic patients with and without hiatus hernia. "Symptomatic patients were readily separated from asymptomatic ones by their weaker base-line sphincter strength and decreased sphincteric response to the stimulus of an increase in intra-abdominal pressure. However, neither in the asymptomatic group nor in the symptomatic groups of patients could any effect of hiatus hernia be found". They concluded that “the rationale for surgical repair of hiatus hernia in patients with gastroesophageal reflux must therefore be questioned”. It took over 15 years before Mittal et al. demonstrated the vital effect of the crural diaphragm on the lower oesophageal sphincter pressure, specifically in relation to increased intra-abdominal pressure, and even longer for Kahrilas et al. to restore the hiatal hernia to its proper place in the pathophysiology of gastro-oesophageal reflux.
Defective motility.

In 1883 Kronecker and Meltzer were the first investigators to study the patterns of oesophageal pressure changes during swallowing by means of small balloons. In 1953 Butin et al. published the first oesophageal pressure measurements by means of an electric transducer, originally intended to record intravascular pressures. The transducer was mounted on the distal tip of gastric tube. They described the “normal swallowing complex” consisting of “an initial wave of negative pressure and three subsequent waves of positive pressure”. The first positive wave was thought to represent the passage of the swallowed material, the others “a distally moving, peristaltic wave of contraction in the esophagus”. In the same year Sanchez et al. published a manometric study focussed on the distal oesophagus. The used two open tipped water filled catheters attached to each other, with their tips 8 cm apart. While arriving at similar results as Butin et al. they could demonstrate the peristaltic nature of the positive wave which they reduced to one by registering dry swallows. Their most significant finding was in the distal oesophagus, in what they termed the vestibule, where they noted the absence of the positive peristaltic wave which led them to suggest “that this distal portion possesses an independent motor function”. In 1956 Fyke et al. and a year later Atkinson et al. were able to demonstrate manometrically the presence of the lower oesophageal sphincter and to measure its strength. But 10 years later Pope concluded that, “the level of pressure recorded from this area has been equated with sphincter competence by most workers. However, other investigators have not found a good correlation between sphincter pressure and the presence or absence of reflux”. This problem was not resolved, with important overlaps in lower oesophageal pressures between normals and patients with reflux oesophagitis still being found by Kahrilas et al. in 1986. However, in addition to the aforementioned overlap, they introduced the factor oesophageal peristaltic dysfunction into peptic oesophagitis. This manifested itself in failed primary peristalsis, i.e. no peristaltic contraction following deglutition or if the peristaltic contraction did not traverse the entire length of the oesophagus. In addition, peristaltic amplitude in the distal oesophagus was significantly lower in oesophagitis, with the degree of peristaltic dysfunction increasing with more severe degrees of oesophagitis. The authors pointed to the link between their findings and the earlier studies demonstrating prolonged acid clearance in reflux oesophagitis. However, it needed the concept of inappropriate complete lower oesophageal sphincter relaxation to advance the problem of correlating sphincter pressures with reflux oesophagitis.

Experimental reflux oesophagitis.

(Note, this section is both detailed and contains some gruesome details, general readers and anti-vivisectionists are advised to skip it)

The first successful attempt at inducing oesophagitis experimentally would appear to have been in 1950 by Ferguson et al. They cited a number of earlier, unsuccessful studies including that by Friedenwald who had failed to induce ulcers by plain HCl without previous mechanical ablation of the mucosa. They employed a wide selection from the animal kingdom including humans (cadavers), dogs, cats, rats, guinea pigs, and hamsters. Gastric juice was brought into contact with the oesophageal mucosa, either by making the animal vomit or regurgitate, or by perfusion with gastric juice from dogs or humans, the former without or after histamine or pilocarpine
stimulation, the latter all after histamine stimulation. The pH values were between 1.2 and 1.7 and 1.4 and 1.9 respectively. In addition, the pylorus was ligated and histamine injected in a number of animals while in others the duodenum was ligated distal to the bile and pancreatic duct. Vomiting was induced by apomorphine, ether anaesthesia or increased intracranial pressure.

As can be imagined, the enormous diversity of experiments produced a large variety of results. However, the final conclusion was that acid gastric juice with peptic activity has a prompt and devastating effect, this in contrast to plain HCl. The addition of bile or pancreatic secretions by ligation of the distal duodenum, actually inhibited the effect of gastric juice78.

A year later, in a rather more focussed study from the same centre, Cross and Wangensteen perfused the oesophagi of cats for a maximum of 8 hours, with canine bile, pancreatic juice, a combination of these two, sodium taurocholate and glycocholate, jejunal juice and human bile. With the exception of jejunal juice, all these fluids caused more or less severe oesophagitis. In dogs, duodeno-oesophagostomies, cholecystojejuno-oesophagostomies, jejunooesophagostomies and total gastrectomies with end to end oesophago-duodenostomies were constructed to cause diversion or regurgitation of bile and pancreatic juice, pancreatic juice or bile alone, and succus entericus into the oesophagus. They were endoscoped at regular intervals and sacrificed after 1-3 months. Bile and pancreatic juice, singly or in combination produced oesophagitis which induced anaemia with guiac positive stools. The presence or absence of the acid secreting part of the stomach and total gastrectomy made no difference, interpreted as excluding gastric juice regurgitation as a factor involved in causing oesophagitis in this model. Only succus entericus and oesophago-antral anastomosis with pyloroplasty (i.e. adequate drainage) failed to produce oesophagitis. An important conclusion was that when total gastrectomy is performed in man, alkaline oesophagitis, caused by regurgitation from the duodenal loop, should be prevented by a Roux-Y-plasty,79 a conclusion which needed to be repeated regularly over the subsequent 25 years80.

In 1959 Redo performed perfusion studies in the canine oesophagus for up to 8 hours, comparing the effects of gastric juice, pepsin, hydrochloric acid, bile and combinations of these. In addition, he examined the effects on various isolated segments of the oesophagus. Both gastric juice and HCl with pepsin at a pH < 2, the latter with an optimum at a concentration of 2%, caused severe erosions and ulceration. After dialysis the effect of gastric juice was enhanced, suggesting an inhibitory factor had been removed. Bile alone had little effect and in combination with pancreatic juice occasionally produced slight erosions. The activity of gastric juice was actually inhibited by bile.

The susceptibility of the 3 segments to the ulcerogenic factors tested was found to be similar81. These three studies confirmed the effects of gastric juice with pepsin but the two later studies on bile and pancreatic juice produced somewhat divergent results. While Cross and Wangensteen in both their chronic dog model and in their 8-hour cat perfusion experiments, recorded severe effects of these two fluids, the Redo canine perfusion study, which also had a maximal duration of 8 hours, found only minor damage from bile and pancreatic juice without HCl79, 81. Both species differences and time scales may have been responsible for these divergent results.

However, in 1972 Henderson et al confirmed the lack of effect of bile alone in dogs by chronic perfusion studies, 4 hours daily over a 21 day period. Perfusates consisted of dog bile, taurocholate, taurodeoxycholate and glycodeoxycholate with and without HCl. The effects were monitored by endoscopy and examination after sacrifice at 21 days. In contrast to the Cross and Wangensteen chronic dog study, severe oesophagitis was only induced by bile and bile-acids
when combined with HCl. The greatest effect was seen from the taurocholate-HCl combination.
In addition, they registered motor defects, specifically of the high pressure zone similar to those
found in patients with hiatus hernia. In these dogs the motor disorder was reversible after the
healing of the oesophagitis.82

In the same year Gillison et al. employed a chronic monkey model in which the distal
oesophagus was resected to create reflux. In addition, bile was either excluded from the stomach
by a Roux-Y procedure or shunted into the stomach by a cholecysto-gastro-stomy, the latter
group including a subgroup subjected to stimulated acid production by regular histamine
injections. The effects were examined by endoscopy at one and three months. Reflux with gastric
juice alone produced only mild oesophagitis; gastric juice contaminated with bile produced more
substantial oesophagitis ranging from mild to ulceration. Histamine stimulation produced no
additional effects83.

These 3 chronic studies, demonstrating the effects of bile and/or pancreatic juice, again produced
divergent results. In the Cross and Wangensteen surgical model no HCl was involved79, in the
Henderson chronic perfusion model the addition of HCl was essential82 and in the Gillison model
there was only a minor role for gastric juice. Here, however, in contrast to the Redo findings, the
activity of gastric juice was enhanced by bile83. Apart from the dangers of alkaline reflux, no
final, clinically relevant, conclusion could be drawn from all of these experiments.

In 1975 Safaie-Shirazi et al. expanded the field of enquiry by addressing the hypothesis of a
“mucosal barrier” to H+, which, when broken, allowed the escape of H+ from the lumen. This
barrier had been shown to exist in gastric mucosa and bile salts were found to have been
responsible for its breaching. They instilled an HCl solution with and without various
concentrations of bile salts in isolated oesophageal segments of dogs. The net ion flux was
determined after 30 minute periods. Bile salts induced a 4-fold higher loss of H+ and Cl- than
plain HCl. Increasing concentrations of bile salts had no additional effect on the ion flux. In
addition, severe necrotizing oesophagitis was caused by the combination of HCl and bile salts. A
similar experiment was performed in humans

where the distal oesophagus was occluded by a Sengstaken-Blakemore tube, bile salts producing
a 5-fold increased H+ loss84. The concept of a mucosal barrier to H+ which could be breached by
bile salts, or possibly, other detergent or enzymatic substances, was established by this study. It
explained the failure, in previous studies, of plain HCl to cause substantial damage to the
oesophageal mucosa.

In 1977 Safaie-Shirazi published another study on the effect of pepsin on ionic permeability.
Using the same technique as before she now instilled various concentrations of HCl and pepsin.
She again observed an increased H+ loss after the addition of pepsin which, however, was
reversed at the highest pepsin concentration. She had demonstrated that pepsin, at certain
concentrations, was also capable of breaching the H+ mucosal barrier85.

In 1980 Kivilaakso et al. published the first of two studies on the effects of potential harmful
agents present in gastric juice and duodenal contents on isolated rabbit oesophageal mucosa in
vitro. Mucosal integrity was assessed by measurements of transmucosal potential differences,
tissue electrical resistance and, when acid was present, permeability to H+. Test substances were
sodium taurocholate, three human deconjugated bile salts, lysolecithin, pepsin, trypsin and
phospholipase A.
Taurocholate at pH 3.5, but not at pH 7.4 and both deoxycholate and Chenodeoxycholate at pH 7.4, produced profound effects. Pepsin in the presence of H⁺, lyssolecithin to a lesser degree and trypsin in the absence of H⁺, produced less profound effects than those caused by bile salts. They concluded that in the presence of gastric acid, pepsin and conjugated bile salts contribute to oesophagitis, but in the absence of acid, trypsin and especially unconjugated bile acids were responsible. Their second study, published in 1982, examined the role of luminal H⁺ on the pathogenesis of oesophagitis in an in vivo rabbit model. An isolated segment of oesophagus was perfused with taurocholate, pepsin and lyssolecithin with and without HCl. The severity of mucosal damage was assessed by transmucosal potential difference, net flux of Na⁺ and two neutral molecules of different sizes, H₂O and C-erythrol. Plain HCl again produced minimal effects, even in unphysiological high concentrations which, although inducing a markedly increased diffusion of H⁺ into the mucosa, had only a minor influence on mucosal integrity. This in contrast to the three tested agents where pepsin caused more extensive mucosal changes than taurocholate, the magnitude of the H⁺ efflux was far greater in the latter, suggesting that the damage caused by pepsin was not mediated by accumulation of luminal H⁺. In fact, the severity of the mucosal damage caused by each of the individual test agent was not dependent on the HCl concentration used. This study would appear to have displaced HCl from its role as a prime mover to that of a facilitator.

From 1982 Lillemoe et al. published a number of studies into the discordance between substances which breached the barrier and those which caused mucosal damage in an in vivo rabbit model. They continuously perfused the oesophagus at pH 2 with pepsin, taurodeoxycholate or trypsin, the barrier was assessed by net fluxes of H⁺, K⁺, glucose, haemoglobin and tritiated water, mucosal damage by gross and microscopic examination. Pepsin caused both increased permeability of the barrier and mucosal damage, taurodeoxycholate also increased permeability but caused no significant pathology while trypsin at pH 2 had no effect whatsoever. A year later they presented a study into the comparative effects of taurodeoxycholic acid, tauroursodeoxycholic acid (TUDC) and taurochenodeoxycholic acid (TCDC) at 3 concentrations on the gastric and oesophageal mucosa, a study prompted by the clinical use of the last two bile acids in the dissolution of gallbladder stones. For the oesophagus the rabbit model was employed, the barrier function being assessed by measurements of transmucosal potential differences, tissue electric resistance and net H⁺ flux. At each concentration TUDC was found to produce far less disruption of the barrier function than either TDC or TCDC. There were no differences in gross and microscopic mucosal damage, which was actually minimal. Their next study repeated the first, but now at a pH of 7.5 to simulate alkaline oesophagitis. Here trypsin caused severe morphologic changes but only minimal disruption of the mucosal barrier, taurodeoxycholate caused extensive disruption of the mucosal barrier but only minimal oesophagitis. Pepsin, at this pH, had no effect. Finally, in 1985, Lillemoe et al. investigated the effects of taurodeoxycholate on the mucosal damage caused by pepsin and trypsin at their optimal pH values of 2 and 7.5 respectively. Surprisingly, TDC reduced the mucosal damage and barrier disruption by pepsin in a dose dependent manner. The damage caused by trypsin on the other hand, was enhanced by TDC, again dose dependently. This inhibition of pepsin activity by bile acids, had already been demonstrated in vitro in several studies by Tompkins et al. Previously, Mud et al from Rotterdam had examined the effects of gastric, biliary and pancreatic reflux in a variety of combinations by means of surgical procedures in a chronic rat model. This had only produced oesophagitis in
those combinations which included pancreatic juice. Oesophageal washouts before and after operation were tested for concentrations of trypsin and bile acids. There were significant differences in the concentrations of trypsin, but not of bile acids, between rats with and without oesophagitis.95

These studies appeared to have established a role for bile acids in breaching the H⁺ barrier with conflicting results on the amount of mucosal damage and Kivilaakso versus Lillemoe. Pancreatic enzymes were found both to breach the H⁺ barrier and cause extensive mucosal damage at their optimum pH. In practice, this optimum pH for pepsin would not often be achieved while in contrast to the Gillison findings, its activity was also inhibited by bile acids. The in vivo Mud model suggested that, in practice, trypsin, the effect of which was enhanced by bile acids, was a prime suspect. The role of HCL now appeared to be reduced to that of creating a suitable pH for pepsin activation.

However, these often contradictory results, which may in part have resulted from species differences, have made it impossible to distill hard and fast conclusions applicable to human reflux oesophagitis. Obvious shortcomings of all these experiments were that the time scale of human reflux oesophagitis could not be measured in hours or weeks, but in months or years. In addition, in clinical practice, all sorts of variations which were as yet not examined would occur in the already complicated relationships between various potentially harmful substances.

On the other hand, it should be stressed that these pioneering efforts certainly produced a number of basic facts on which clinicians should build in analysing the mechanism of reflux oesophagitis. These are, for instance, the fact that the oesophagus is well protected against acid and this protection needs to be breached by bile acids and/or enzymes, substances which by themselves are also able to cause substantial damage to the oesophageal mucosa. The unexpected interaction between bile acids and pepsin add an extra dimension to this already very complicated patho-physiological conundrum. Such studies would, in the first place, need to accommodate the empirical finding that reflux oesophagitis heals under strong gastric acid inhibition which would tend to point towards enzymes and bile acids active at a low pH.

Clinical studies.

In 1953 Aylwin was the first to examine the oesophageal juices just above the cardia in 50 patients with hiatal hernias in whom the degree of oesophagitis had been established, ranging between none to stricture. The juices were collected by suction through a thin polythene tube while the patients were asleep. There was a clear relationship between the degree of oesophagitis, a pH under 4 and the concentration of pepsin in the collected juice, although in strictures the results were influenced by collections of saliva. He was surprised to find no secretion in 13/19 patients without oesophagitis, concluding that they had no incompetence of the cardia at night, possibly through the presence of a sphincter.

As there were no signs of reflux from the stomach, Aylwin assumed that the refluxate had originated from the herniated pouch under vagal influence. He also emphasised the roles of saliva and oesophageal gland secretions in protecting the oesophagus and pioneered the concept of nocturnal reflux in oesophagitis.98

As the results of experimental oesophagitis became available, interest shifted from acid to bile and pancreatic juices. These were obviously less easily detected. Gillison et al found a strong correlation between symptoms of heartburn and the regurgitation into the stomach of barium which had previously been instilled into the duodenum through a tube.99 By aspiration of gastric juice from the proximal stomach after a liquid meal Kaye and Showalter demonstrated that the
post-prandial bile-salt concentrations in juice from patients with symptomatic gastro-oesophageal reflux were higher than those of normal controls\textsuperscript{100}. At the same time two studies were published involving a young surgeon from Rotterdam, Dick Stol and addressing the acid output and bile acid concentration in gastric juice from patients with oesophagitis. The first study compared patients with hiatal hernias and various degrees of oesophagitis with normal controls. Acid output was measured before (basal) and after (peak acid output) pentagastrin stimulation\textsuperscript{101}. Basal and peak acid outputs were marginally higher in patients than in controls while the mean bile acid levels were significantly higher with, however, a very considerable spread and a considerable overlap with controls. There was a tendency towards the combination of more severe degrees of oesophagitis with higher acid output and bile acid concentrations although it was notable that only 4 of 32 patients had a higher peak acid output above the locally accepted normal value\textsuperscript{102}.

The second was a repeat of the previous experiment with the addition of a test meal. The results were disappointing, as the result of 4 outliers the patients had higher mean bile acid concentrations after the test meal than controls and the peptic stricture patients again had a higher basal acid production\textsuperscript{103}. However, these studies of acid output and gastric bile contamination in a static situation were unable to identify the distinguishing factors between individuals with and without reflux oesophagitis. Extending the observation time by 24-hour ambulatory monitoring of both oesophageal pH\textsuperscript{104, 105} and bile reflux\textsuperscript{106, 107} were necessary in setting the first steps towards this goal.
The Diagnosis of Reflux Oesophagitis.

Radiology.

As mentioned previously, radiology was one of the founding disciplines for the concept of the hiatal hernia and the main pioneers have been summarised. However, in radiology the link between the hiatal hernia, gastro-oesophageal reflux and oesophagitis had not yet been established. This changed with a study published in 1953 by Flood et al. who examined the relationship between hiatus hernia, insufficiency of the cardia, acid reflux and oesophagitis. They had been impressed by a number of English studies emphasising the regurgitation of gastric contents into the oesophagus and that this was an important mechanism in the aetiology of oesophagitis. Their patients were routinely examined in supine, Trendelenburg and prone positions, were asked to strain and pressure was applied to the abdomen. The most successful manoeuvre for demonstrating the hernia was found to be the act of turning over from the supine to the prone position. Barium reflux into the oesophagus was found in 18 of 34 patients with a hiatus hernia. No cases of reflux were found in a control group of patients without hiatus hernia, subjected to the same routine. Only 2 cases of reflux were observed in 100 patients undergoing routine gastrointestinal x-ray series. They also tested for reflux in patients in the right recumbent position, by passing a 12 French stomach tube after histamine stimulation of the gastric acid production and aspirating fluid at 5 cm intervals from 30 cm they attempted establish acid reflux. Free acid found at 30 cm below the gum margins (obviously few patients with incisors) probably signified oesophageal reflux. Although the correlation between the endoscopically established diagnosis of oesophagitis and this early reflux test was understandably tenuous, this study did signal the shift from the simple radiological diagnosis of hiatal hernias to the presence of reflux.

In 1966 radiologists, by employing manometry and cine-radiography managed to distinguish between the lower oesophageal sphincter, the A ring, and the B ring representing the hiatus.

In spite the good results of double contrast radiography and snide remarks by Meyers about the dangers of fibre-optic endoscopy, the general availability of endoscopy heralded the gradual disappearance of radiology from the diagnostic menu of reflux oesophagitis.

Endoscopy

Reflux oesophagitis was first described on the basis of endoscopic observations and consequently endoscopy has remained the mainstay in diagnosing this condition. However, it was only after the introduction of fibre-optic endoscopy that this technique was able to establish its current dominant position. Important adjuncts were oesophageal biopsies examined according to the Ismail-Beigi criteria. A classification of different degrees of reflux oesophagitis according to Savary Miller was published in 1987.
Manometry

In 1962 Code et al. developed a manometric test for hiatal hernia using an array consisting of a pressure transducer in a tube over which a tiny balloon filled with water was fitted and three water filled polyethylene catheters with lateral orifices. By identifying the hiatus as the point of “pressure respiratory reversal” and the lower oesophageal sphincter, which was normally found 2-3 cm below the hiatus by the balloon-covered transducer, manifested by a zone of elevated pressure 3-5 cm in length. A double respiratory reversal as the detecting units traversed the junctional region was found to be the most significant indicator of a hiatal hernia.

Acid perfusion and pH monitoring

In the absence of easily accessible endoscopy, the acid perfusion test, introduced by Bernstein in 1958, provided a useful diagnostic tool for oesophagitis. In this test a tube was introduced through the nares over a distance of 30-35 cm in the oesophagus. Subsequently test solutions of 0.1N HCl or a control solution of 0.9% NaCl were randomly administered over 30 minute periods. The test was positive if it elicited persistent and often progressive symptoms while control solutions never caused symptoms. The pain was found to be projected to a wide variety of locations on the chest and upper abdomen. In his test population Bernstein had established the presence of oesophagitis by oesophagoscopy. The test was false negative in one case of oesophageal ulcer; however he found apparently false positives in 10/12. He called this condition “pseudoesophagitis”, and was the first to describe what is now termed “non-esophagitis reflux disease” or “NERD”.

A further refinement was introduced by Tuttle et al. who combined the acid perfusion test with simultaneous measurement of intraluminal pressure and pH. After removal of the tube for the acid perfusion test a glass pH electrode and a water filled tube for measuring pressure changes, with its orifice at the level of the bulb of the pH electrode, were introduced into the oesophagus through the nares. This array was passed into the stomach and then withdrawn at 1 cm at a time. The pressure inversion point was identified and acid regurgitation diagnosed when a pH of 4 or less was encountered at least 4 cm above the pressure inversion point. In 105 of 124 patients there was concordance between the two parts of the test and in 15 the acid perfusion moiety was negative while acid regurgitation was positive, a discrepancy which was thought to have resulted from antacid therapy which might have healed their oesophagitis.

Another indirect test was the “Acid clearance test from the distal esophagus” introduced by Booth et al. in 1968. Here a pH probe and 3 joined polyvinyl tubes, with their open tips at 5 cm intervals, were passed through the nares, with the pH probe placed level with the most distal opening, 5cm above the distal oesophageal sphincter. Next 15 ml of 0.1N HCl was injected through the most proximal tube and the number of swallows needed to restore the pH to 6.0 counted and when this pH had been achieved, reflux was tested by the Müller and Valsalva manoeuvres and a cough. A fall in pH to below 4.0 was considered positive evidence of reflux.

In 1964 Miller et al. noted that hiatal hernias can be demonstrated in 10% of asymptomatic persons over the age of 40 while a study by Eyring had found 43% of patients with demonstrable hiatal hernias to be asymptomatic. On the other hand, no hiatal hernia was demonstrated in 21% of patients with endoscopically verified oesophagitis. The then apparently current policy
of operating on hiatal hernias and the high recurrence rates led the authors to conclude: “Diagnosis is, of course, of the first order of magnitude, but after having established the presence of an anatomical defect, the important question that then arises is, “is surgery indicated?” They described a technique for measuring oesophageal pH for up to 24 hours. In controls the pH in the terminal oesophagus never fell below 6.5, however, in a patient without a hiatal hernia, pH values as low as 2 were found and oesophagitis endoscopically confirmed. The authors speculated that “this method may be very useful in establishing the diagnosis of esophagitis, especially when the radiological examination is within normal limits”.

However, it took another 10 years before dissatisfaction with the acid perfusion test led Johnson and Demeester to achieve this goal by means of their twenty-four-hour pH monitoring of the distal oesophagus. They employed a Beckman gastric pH probe positioned 5 cm above the distal oesophageal sphincter as determined by infusion manometry and the measurements were recorded on a strip chart recorder. The essential feature was their scoring system of six components, comprising the percentage of time with the pH below 4 over the 24-hour period, and separately for the periods in supine and upright position, the total number of single reflux periods, the number of episodes greater than five minutes and the time of the longest reflux episode. Their technique, in combination with their scoring system and later expanded to 24-hour ambulatory pH monitoring, became the standard for the next three decades and made possible both the definition and ascertainment of gastro-oesophageal reflux disease or GORD.

Interestingly, the pH 4 cut off point was derived from a study by Tuttle who had found the onset of pyrosis to occur at this pH. As this study was performed in a military hospital it is likely that both their “normal” volunteers and symptomatic patients were relatively young and the applicability of their data to elderly subjects could be challenged. This challenge was presented in 1989 by Smout et al. who, employing an ambulatory combined oesophageal pH and motility recording device, examined 32 healthy volunteers, 16 under and 16 over the age of 45. A comparison of the results obtained from the two age groups, revealed significantly higher values in the over 45 group for both time pH< 4 and for the number of episodes> 5 minutes during the upright and total 24 hour periods. In addition, they found the number of simultaneous contractions and the mean duration of the perprandial peristaltic contractions to increase with age. Unfortunately, the volunteers had not undergone endoscopy so the integrity of their oesophagus was unknown.

This challenge was subsequently rejected by Richter et al., including DeMeester, who examined the influence of study centre, pH electrodes, age and gender. They pooled the results from three study populations, totalling 110 asymptomatic healthy paid volunteers, who had undergone ambulatory 24-Hr oesophageal pH studies. Of the four factors examined, the influence of the first two was found to be negligible. Males generally tended to have more oesophageal reflux than women and men over 50 actually experienced significantly more reflux episodes > 5 minutes. The gender difference was attributed to the greater acid secretory capacity of males. The conclusion was that the existing normal values were still applicable but consideration should be given to developing separate standards for men and women.

One year later Fass et al. published a study: “Age-and gender-related differences in 24-hour esophageal pH monitoring of normal subjects”. They studied 30 asymptomatic volunteers, 15 <65 years and 15 ≥ 65 years and 15 males and females. They found considerable differences between the younger and the older group which were not statistically significant, probably as the result of the too small number of subjects. On the other hand there were significant gender
differences with all pH variables, except episodes > 5 minutes, lower in females. These results suggested a need for redefining sex-specific normal 24-hr pH monitoring, a suggestion which however, does not appear to have been acted upon. Another worrying finding was that 30% of their asymptomatic volunteers had abnormal results, raising the question of the frequency of “silent refluxers”, an issue which was to resurface in the epidemiology of Barrett’s oesophagus.

**Treatment of reflux oesophagitis.**

Before the introduction of H₂ receptor blockers and proton pump inhibitors the medical treatment of severe reflux oesophagitis was quite limited and had not changed much from the days of Arthur Hurst. In 1973, John Bennett, a foremost British specialist on reflux oesophagitis gave an overview of medical treatment. The cornerstone was antacid, hourly doses to begin with, possibly in combination with metoclopramide. Patients should stop smoking and if overweight, lose weight. The head of the bed should be propped up 8 inches. However, if medical treatment failed or there were serious complications such as strictures, not responding to repeated dilations or bleeding, surgery was indicated. A problem remained that many elderly patients requiring surgery were considered unfit to undergo an operation.

An effective treatment for ulcers in Barrett’s oesophagus, developed in Rotterdam, was a continuous mid-oesophageal drip with a milk-antacid mixture which cleared up ulcers within a few weeks. It was responsible for a large number of referrals, a number of whom were subsequently included in the Rotterdam Barrett’s oesophagus follow up cohort.

It was not until the introduction of H₂ receptor blockers in 1978 and more especially proton pump inhibitors in 1986 that effective medical treatment of reflux oesophagitis became possible.
The Present State of Reflux Oesophagitis, Barrett’s Oesophagus and Adenocarcinoma of the Oesophagus

The continuity, which over the past 40 years, has been established between reflux oesophagitis, Barrett’s oesophagus and adenocarcinoma, implies that it represents a classical cascade from normal tissue to cancer or, as is technically known, multistage carcinogenesis\textsuperscript{128, 129}. However, this field has become so complicated that a detailed review is beyond the scope of a non-specialist. Therefore this non-specialist has chosen to present a superficial overview of the three subjects in the title and eased the burden by mainly relying on expert reviews.

In a recently published seminar on reflux oesophagitis the pathophysiology section is mainly concerned with the factors influencing the function of the lower oesophageal sphincter\textsuperscript{130}. There are, however, only 3 references to chemical factors, a paucity which may well reflect a certain lack of interest in this branch of pathophysiology now that effective acid suppression by proton pump inhibitors has largely solved the problem of medical therapy without, apparently, creating much interest in its mode of action. Chemical factors are obviously essential in understanding the process of mucosal damage to the squamous cell lining of the oesophagus resulting in metaplasia to Barrett’s oesophagus. Both the supporters of Barrett’s oesophagus originating from oesophageal gland ducts\textsuperscript{131} and those who consider these organs to be irrelevant\textsuperscript{40} could presumably agree with this premise. The change from a squamous cell to a cylindrical cell mucosa obviously implies changes in gene expression and consequently, the molecular phenotype.

Barrett et al. employing oligonucleotide-based micro arrays, characterised gene expression profiles in the three normal upper gastrointestinal mucosae, obtained by endoscopic biopsy and, in addition, including Barrett’s oesophagus. They hoped to identify disease specific genes in Barrett’s oesophagus and thus gain insight into the molecular basis of early neoplasia. They cited earlier studies which had shown that the development of Barrett’s metaplasia and the subsequent evolution of neoplasia were associated with the inactivation of the CDKN2A/p16 gene and the expansion of clonal populations of epithelial cells. In the event they found clear distinctions between the expression profiles of the oesophageal, gastric and duodenal tissues of the upper GI tract, and were thus able to identify with clusters of 100-200 genes, specific to each tissue. Although Barrett’s oesophagus shared extensive transcriptional similarities with all three, it did manifest a separate cluster of 38 specifically up-regulated genes. A lineage-specific developmental association with any one of the surrounding tissues was not demonstrated. Barrett’s oesophagus did contain trefoil peptides, key mediators of initial restitution of damages mucosa in the GI tract but absent in squamous epithelium. In addition, the 38 up-regulated genes in the Barrett’s oesophagus cluster also included genes which are associated with a number of different pathways including cellular migration, alterations in the cell cycle, apoptosis and stress responses, all of which have been associated with neoplasia\textsuperscript{132}.

In 2005 Brabender et al. claimed to have developed a technique of gene expression profiling by means of a panel of highly selected genes by which they were able to distinguish
between patients with Barrett's oesophagus, patients with Barrett's-associated adenocarcinoma, and a healthy control group from endoscopic biopsies taken from the normal squamous oesophageal epithelium above the pathological areas. They concluded that they had defined the existence of a carcinogenic field effect. Recently Wang et al. performed transcriptional profiling on tissues from patients with a normal oesophagus, with Barrett's oesophagus, subdivided into patients with and without concurrent adenocarcinoma of the oesophagus and adenocarcinoma of the oesophagus. 457 genes were significantly differentially expressed in adenocarcinoma of the oesophagus versus normal oesophagus against 295 between Barrett's oesophagus and normal oesophagus. However, only 36 genes were differentially expressed between Barrett’s oesophagus and adenocarcinoma of the oesophagus. Finally Barrett’s oesophagus and adenocarcinoma of the oesophagus shared 212 genes differentially expressed from normal oesophagus. The authors concluded that Barrett’s oesophagus is biologically closer to adenocarcinoma of the oesophagus than normal oesophagus. They also found 12 genes which were significantly differentially expressed between Barrett’s oesophagus with and without concurrent adenocarcinoma of the oesophagus and suggested that these 12 genes could be potential biomarkers for diagnosing adenocarcinoma of the oesophagus in an early stage.

So, given the fact that considerable genetic changes occur in the changes from normal oesophageal mucosa to adenocarcinoma of the oesophagus, can any system be discerned?

Hanahan and Weinberg, in a review titled “Hallmarks of cancer”, discussed the rules that govern the transformation of normal human cells into malignant cancers. They suggested “that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth. 1: self-sufficiency in growth signals, 2: insensitivity to growth-inhibitory (antigrowth) signals, 3: evasion of programmed cell death (apoptosis), 4: limitless replicative potential, 5: sustained angiogenesis, and 6: tissue invasion and metastasis”. (see figure)

These six items returned in a subsequent reviews by Souza and Spechler which, although focussing on the prevention of adenocarcinoma of the distal oesophagus and proximal stomach, provided an overview of the best known mechanisms involved in each of the six steps.

The first, self-sufficiency in growth signals or the independence from exogenous mitogenic stimulation or inhibition results in the increased rate of proliferation in tumour cells and is attributed to inactivation of the retinoblastoma protein (Rb) which normally controls the progression through the cell cycle at the restriction or R point. The inactivation of Rb is mediated through oncogenes. Normal cellular genes promoting cell growth are, somewhat pejoratively, termed proto-oncogenes, only becoming oncogenes after mutation. Cyclin D1 is such an
oncogene; it interacts with cyclin-dependent kinases which in turn are responsible for inactivating Rb through phosphorylation. Cyclin D1 is found over expressed in Barrett’s oesophagus. This in contrast to Cyclin B1, which is involved in the G2 to Mitosis transition in the cell cycle and is only found in dysplastic Barrett's oesophagus and adenocarcinoma of the oesophagus. Another pathway consists of alterations to the growth factors, growth factor receptors or the signalling pathways that mediate growth factor-receptor interactions. Ras proteins, which have Cyclin D1 as its downstream target, can be activated by growth factor receptors of the tyrosine kinase family. Increased expression of epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) have been found in adenocarcinoma of the oesophagus while increased levels of both the EGF receptor, EGFR and TGF-α have been found in Barrett's oesophagus.

The second step comprises the inactivation of tumour suppressor genes such as p16 and p53, which normally block Rb phosphorylation. This inactivation can be caused by mutation, deletion of the chromosomal region containing the gene, termed loss of heterogeneity, LOH, and promoter methylation. Over expression of MDM2, an inhibitor of p53 is sometimes found in those adenocarcinomas of the oesophagus where wild type p53 is still present, thus forming an alternative p53 inactivation mechanism to mutation. Inactivation of the adenomatous polyposis coli or APC gene by LOH has also been observed in both Barrett's oesophagus and adenocarcinoma of the oesophagus.

The third step, evasion of apoptosis, can be achieved by inactivating p53 but also by the turning the tables on lymphocytes on the Fas with Fas-ligand (Fasl) death receptor pathway. Normally, Fasl on activated lymphocytes bind to the Fas receptor of the target cell, inducing apoptosis. However, adenocarcinoma of the oesophagus cells express Fasl which after binding to the Fas receptor on the attacking lymphocytes induce their apoptosis. Another escape route is the synthesis of an apoptosis blocking agents such as cyclooxygenase-2 (COX-2). Over expression of COX-2 has been detected in Barrett's oesophagus, with increasing expression during the progression to dysplasia and adenocarcinoma of the oesophagus.

Step 4, involves limitless replicative potential or resistance to cell senescence. Senescence is the mechanism by which the capacity of normal cells to proliferate is limited by the shortening of telomeres, stretches on non coding DNA situated at the ends of the chromosomes, which occurs at every cell replication. Once the telomeres have been reduced to a critical length the cell will exit the cell cycle into a G0 state entailing permanent growth arrest. In order to escape this mechanism and become immortal, malignant cells need to maintain their telomere length. This they achieve by means of telomerase, an enzyme which adds telomeric sequences to the ends of chromosomes. Telomerase is not found in normal somatic cells; low levels of expression are found in non dysplastic Barrett's oesophagus with increasing levels in the transition from low- to high-grade dysplasia and the highest expression in adenocarcinoma of the oesophagus.

Step 5, continual angiogenesis or the formation of new blood vessels, is obviously essential for the sustenance of the rapidly growing mass of tumour cells. The process is stimulated by the binding of vascular endothelial growth factors (VEGFs) to their tyrosine kinase receptors or VEGFRs which then initiate signalling pathways causing the proliferation and migration of endothelial cells. Compared to Barrett's oesophagus, VEGF mRNA and protein expression is only significantly increased in adenocarcinoma of the oesophagus, because even dysplastic Barrett's mucosa is not yet in need of angiogenesis.

The final step 6, tissue invasion and metastasis by tumour cells, is thought to involve abnormalities in cell to cell connections. Catenins α, β, and γ are cytoplasmic proteins attached to
the cytoskeleton to which cell adhesion molecules named cadherins are anchored. The best known combination is the E-cadherin-catenin complex. Failure of this interaction impairs cell adhesion, resulting in invasion and metastasis. In addition to cell adhesion, β-catenin is also involved in cellular signal transduction by stimulating the production of genes promoting cell growth. While in squamous oesophageal mucosa and non dysplastic Barrett's oesophagus both E-cadherin and β-catenin are mainly found in the cell membrane, with increasing degrees of dysplasia membrane staining for these proteins decreases while an increase is seen in cytoplasmatic and nuclear staining. Significantly more nuclear accumulation of β-catenin was found in adenocarcinoma of the oesophagus than in that of the gastric cardia. A second group of players in this field are the matrix metalloproteinase’s (MMPs) who thank their name to their membership of a family of zinc dependent proteolytic enzymes involved in the destruction of the extracellular matrix. MMP-7 or matrilysin has been found to be the principle MMP in both Barrett's oesophagus and adenocarcinoma of the oesophagus.

Very recently Maley published a mini-review on “Multistage carcinogenesis in Barrett’s esophagus” in which he approaches the sequence of Barrett's oesophagus to adenocarcinoma of the oesophagus from a slightly different angle. In this he criticises the description of multistage carcinogenesis “as a deterministic and linear series of lesions”. Instead he introduces an illustrated scheme of neoplastic progression in Barrett’s oesophagus to cancer, see figure below.

Figure. An example of neoplastic progression in Barrett's esophagus. Frequency in the neoplasm is shown on the Y-axis and time is on the X-axis. Clonal expansion along the Y-axis (frequency) with time represents clonal expansion in the two-dimensional surface of the BE epithelium. Clones evolve within a neoplasm through processes of mutation, natural selection and genetic drift that appears to take decades. Evolutionarily neutral mutations will randomly increase and decrease in frequency unless they occur in a cell that also has a selectively advantageous mutation. In this case they are called ‘hitchhikers.’ Hitchhikers may occur before (hitchhiker 1) or after (hitchhiker 2 and 3) the occurrence of the advantageous mutation, as long as they arise before the selective sweep is complete. Loss of each allele of CDKN2A (pl6) provides a selective advantage to the mutant clone and drives a selective sweep of that clone through the neoplasm. Lesions in TP53 appear to only expand in the background of a CDKN2A lesion and are thought to be associated with chromosomal instability. Aneuploid clones typically arise within TP53- clones and oesophageal adenocarcinomas are usually aneuploid. Noting that the “initiation of Barrett’s oesophagus remains a mystery”, Maley advances 3 hypothetical mechanisms for this initiation. The first is based on the clear association with gastro-oesophageal reflux, “so BE may just be the result of altered differentiation of esophageal epithelium due to the reflux environment”. After citing the study by Barrett et al. mentioned above, he highlighted the attention now focused on the homeobox genes CDX1 and CDX2, with CDX2 being found in practically all Barrett’s oesophagus samples and even in reflux oesophagitis. The role of CDX2 in Barrett’s oesophagus was recently addressed by Moons.

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et al. from Rotterdam, who found it to be strongly associated with the presence of specialised intestinal metaplasia, which, in view of its role in the development and differentiation of intestinal epithelium, was not unexpected. Predictably, it was not found in gastric metaplastic epithelium or reflux oesophagitis. However, low levels of CDX2 mRNA were found in a number of squamous epithelial biopsies taken 5 cm above the squamo-columnar junction in patients with Barrett’s oesophagus.

The second hypothesis, the migration of columnar epithelium from the cardia is dismissed by Maley on the basis of the Gillen study, while still allowing the possibility of the oesophageal gland duct hypothesis. His third and presumably favoured hypothesis is “a mutation which confers a competitive advantage on the mutant clone”. He considers the best candidate mutation to be the loss of the CDKN2A/p16 tumour suppressor gene, as alterations to this gene are found in 85% of Barrett’s oesophagus patients at first endoscopy, possibly because these cells may enjoy a selective advantage in the reflux environment. As mentioned earlier, this loss can result from loss of heterozygocity, LOH, hypermethylation or sequence mutations. The 15% without this mutation, or the larger group where it is not present in all biopsies, may be the result of mutations in other loci of the CDKN2A/p16 pathway.

The first important genetic alteration in Barrett’s oesophagus is therefore CDKN2A/p16. Its inactivation in selective sweeps breaches the tumour suppressive mechanisms of crypt structured tissue architecture which eventually leads to the spread of mutant clones across hundreds of thousands of crypts. The next to be inactivated is TP53. The loss of TP53, which in Barrett’s oesophagus only occurs after the loss of CDKN2A/p16, carries a 16-fold risk of progression to adenocarcinoma of the oesophagus. In addition, the larger the clone with TP53 LOH, the greater the risk of progression. The loss of TP53 is likely to be responsible for three developments, the first two being the suppression of apoptosis and the prevention of cell cycle arrest, which potentially provide sources of competitive advantage for a clone. The third involves the permitting of genetic instability, which may increase the generation of viable genetic variants. Further events in the progression to adenocarcinoma of the oesophagus are tetraploidy and aneuploidy, both of which are associated with about 10-fold increased cancer risk and may result from the loss of TP53. Senescence via the erosion of telomeres, which constitutes a potential cancer suppressive mechanism, may be circumvented by telomerase, the activity of which is high in adenocarcinoma of the oesophagus although it is unknown whether there is a specific timing during progression or it can be activated at any point. Whether neo-angionogenesis is already activated in Barrett’s oesophagus, as it is in adenocarcinoma of the oesophagus, is unknown, Maley apparently having missed the study by Lord et al. who found the angiogenic factors vascular endothelial growth factor and basic fibroblast growth factor clearly upregulated in adenocarcinoma of the oesophagus but also, to a lesser extent, in some dysplastic Barrett's oesophagus specimens and in goblet cells. Invasion and metastasis may depend on the disruption of E-cadherin, the expression of which has been found to decrease during the progression from squamous epithelium to Barrett’s oesophagus and adenocarcinoma of the oesophagus.

A similar review by Fitzgerald devotes attention to genetic aspects. She notes that analyses of various pedigrees of families with multiple members suffering from heartburn, Barrett’s oesophagus or even adenocarcinoma of the oesophagus, suggest either autosomal-dominant patterns of inheritance with variable degrees of penetrance or an autosomal-recessive inheritance. While the great majority of cases of Barrett’s oesophagus and adenocarcinoma of the oesophagus are sporadic, she cites several lines of evidence for a underlying genetic susceptibility such as
familial clustering and the observations that only a subset of patients with reflux symptoms
develop Barrett’s oesophagus and that neither the degree of reflux exposure nor a previous
diagnosis of reflux oesophagitis are accurate predictors for the development of Barrett’s
oesophagus. Fitzgerald also highlights the potential role of genomics, by employing microarray
technologies, both for uncovering previously unidentified genes predictive of cancer
development and for gene expression signatures predictive of cancer progression.
Adenocarcinoma of the oesophagus which arises in Barrett’s oesophagus resulting from reflux
oesophagitis is a very clear example of an inflammation induced tumour.
This subject was comprehensively reviewed in a paper named: “Inflammation and Cancer, back
to Virchow” by Ballkwill and Mantovani, in which they explained that the response of the body
to a cancer is not a unique mechanism but has many parallels with inflammation and wound
healing, a fact already mentioned in the discussion of proto-oncogenes. The inflammatory
microenvironment of tumours is characterised by the presence of host leucocytes both in the
supporting stroma and in tumour areas. Such tumour infiltrating lymphocytes may actually
contribute to cancer growth and spread, and to the immunosuppression associated with malignant
disease. Three types of leucocytes are discussed. The first, tumour-associated macrophages
(TAM) are a major component of the infiltrate of most, if not all, tumours. TAM are derived
from circulating monocytic precursors, and are directed into the tumour by chemoattractant
cytokines called chemokines. Many tumour cells also produce cytokines called colony-
stimulating factors that prolong survival of TAM. Although, when appropriately activated, TAM
can kill tumour cells or elicit tissue destructive reactions centred on the vascular endothelium,
TAM also produce growth and angiogenic factors as well as protease enzymes which degrade the
extracellular matrix. Hence, TAM can stimulate several of the 6 cancer hallmarks, tumour-cell
proliferation, promote angiogenesis, and favour invasion and metastasis. The second cell type,
dendritic cells, normally has a crucial role in both the activation of antigen-specific immunity
and the maintenance of tolerance, providing a link between innate and adaptive immunity.
However, tumour-associated dendritic cells (TADC) usually have an immature phenotype with a
defective ability to stimulate T cells and consequently TADC are probably poor inducers of
effective responses to tumour antigens. The third type, lymphocytes, or tumour-infiltrating T
cells, predominantly consists of the memory phenotype with only few natural killer cells. The
former produce interleukins associated with a Th-2 response which is ineffective against
tumours. The inflammatory cytokine network which results from the activities of these cells is
then shown to be involved in all six stages of carcinogenesis.
Fitzgerald was the first to demonstrate the qualitatively and quantitatively differences in the
immunoregulatory environment of reflux oesophagitis and Barrett's oesophagus, the latter being
characterised by a distinct Th-2 predominant cytokine profile compared with the
proinflammatory nature of reflux oesophagitis. This finding was recently confirmed by Moons
et al. from Rotterdam. A review from Rotterdam by Bax et al. describes the various
inflammatory mediators, such as cytokines and chemokines, which attract inflammatory cells to
tissues damaged by gastro-oesophageal reflux. NF-κB a transcription factor, is not only involved
with the upregulation of pro-inflammatory cytokines and chemokines, but has also been linked to
oncogenic functions such as proliferation, metastasis and angiogenesis. Activated NF-κB is
found in about half the specimens of Barrett's oesophagus and in most of adenocarcinoma of the
oesophagus. In cell culture NF-κB has been found to be activated by deoxycholic acid. NF-κB in
turn activates the pro-inflammatory chemokine interleukin-8 (IL-8) which attracts neutrophils, the
most potent producers of reactive oxygen species. Deoxycholic acid again induces the expression
of IL-8. IL-8 expression together with that of cytokine IL-1β is increased in Barrett’s oesophagus, especially near the squamocolumnar junction. On the other hand a paradoxically increased expression of the anti-inflammatory cytokines IL-4 and IL-10 has been observed in the distal part of Barrett’s oesophagus where adenocarcinoma of the oesophagus is most commonly found. Besides IL-8 the calprotectin complex is also active in the chemotaxis of neutrophils and both subunits, calgranulin A and B have been found associated with the development of high grade dysplasia in Barrett’s oesophagus, implicating both neutrophils and calprotectin in the neoplastic progression.

Another important player is cyclooxygenase-2 (COX-2), the rate limiting enzyme for the conversion of arachidonic acid into prostaglandins such as prostaglandin E2 (PGE2). PGE2 is currently a much maligned substance, being associated with the inhibition of apoptosis, increasing proliferation and angiogenesis and the induction of metalloproteinase’s. However, it should be remembered that prostaglandins (PGs) first entered the literature because of their key role in protecting the gastric mucosa against injury caused by a variety of necrotizing agents154–157. In a recent survey Gudis and Sakamoto highlighted the good work performed by PGE2, writing: “We think that PGE2 released by COX-2-expressing gastric fibroblasts plays a pivotal role in VEGF production, angiogenesis, and subsequently, the ulcer repair process in gastric tissue”158. The PGE2 story forms a clear example of how normal physiological mechanisms are harnessed to malignant pathways.

Cyclooxygenase-2 itself can be induced by pro-inflammatory cytokines such as TNFα and TGF-β and by growth factors. Cyclooxygenase-2 expression is increased ex-vivo by both bile salts and acid and its expression is considerably in Barrett’s oesophagus and adenocarcinoma of the oesophagus and thus becomes a prime suspect in oesophageal carcinogenesis.

Having established a number of inflammatory mechanisms which are involved in the process of carcinogenesis, it is now necessary to return to the factors causing reflux oesophagitis, the condition which lies at the base of this inflammation.

The technique of ambulatory 24-hour oesophageal pH and bile reflux monitoring106 has clarified some of the questions raised by the pioneers in the field. The static concept of the lower oesophageal sphincter (LOS) pressure determining the degree of reflux has now been replaced by the more dynamic concepts. LOS pressure has been found to vary in relation to phases of the migrating motor complex159. Transient LOS relaxations (TLOSRs) occur spontaneously without a previous swallow and may be the means by which gas can escape from the stomach. They are commonly considered a physiological mechanism160 although there have been attempts to implicate TLOSRs in the aetiology of reflux oesophagitis161. Currently this association is no longer popular162. A study by Allen et al.163 stressed the importance of effective oesophageal clearance of refluxate.

This opinion was underlined by Meneghetti et al. in a study of 827 patients with gastro-oesophageal reflux disease, confirmed by ambulatory pH monitoring. They were divided into three groups on the basis of endoscopic grading of their mucosal injury. Group A had no visible oesophagitis, group B oesophagitis grades I through III and group C Barrett’s oesophagus. All were subjected to oesophageal manometry. As expected, there were significant differences between the three groups as far as the mean DeMeester score was concerned, although there were also very considerable overlaps between all 3 groups. The motility study confirmed older studies indicating that as mucosal damage increased, both oesophageal motility and acid clearance worsened, the latter related to progressive oesophageal dysmotility expressed in both decreasing distal oesophageal amplitude and LOS pressure. As even in the group A patients,
13% had ineffective oesophageal motility and because medical healing of oesophagitis has not been found to result in significant improvement peristaltic function, the authors conclude that the reflux induced mucosal injury extends into the muscular wall. A fact which the late professor van Houten in Rotterdam taught over 30 years ago, stating that in severe oesophagitis the whole oesophageal wall had become thickened and completely fibrotic. The authors then raised the chicken and egg question concerning the natural history of gastro-oesophageal reflux disease, citing the two rival hypotheses. The first, that there are 3 distinct non-communicating categories of gastro-oesophageal reflux disease, non-erosive GORD, erosive GORD and Barrett's oesophagus, based on separate degrees of abnormality of foregut motility. The second hypothesis, which would appear to be more compatible with their own observations, being that GORD should be considered a continuum, with a vicious circle of reflux causing mucosal damage followed by motility disturbances resulting in more reflux. After side stepping this issue, the surgical authors arrived at the, for them, happy conclusion that a laparoscopic fundoplication should be performed early in the course of the disease, an operation which in addition, eliminates the hiatal hernia.

This resurgence of the relevance of the old surgical bugbear, the hiatal hernia, had already been initiated by Kahrilas in 1999 and was recently confirmed in a study from Utrecht where in 16 patients with a small hiatal hernia (3 cm), prolonged high-resolution manometry was performed and both acid and weakly acidic reflux episodes were detected by means of pH-impedance monitoring. Even in these patients, with small hiatal hernias, spatial separation of the diaphragm and LOS in the nonreduced state resulted in a 2-fold increase in acidic and weakly acidic reflux, due to mechanisms other than transient LOS relaxation. There also appears to be a hierarchy of reflux within the group of patients with Barrett's oesophagus. A study by Öberg et al. examined 556 patients by stationary motility, ambulatory pH monitoring and endoscopy with biopsy. After having found cardiac type mucosa in the oesophagus of 411 patients, they then assessed the length of intestinal metaplastic mucosa within the cardiac type mucosa, which resulted in 3 categories, intestinal metaplasia limited to the gastro-oesophageal junction, involving <3cm or >3cm of the oesophagus. An increasing length of intestinal metaplasia was found to correlate with worsening sphincter function, acid clearance and increased acid exposure.

Having found both the motility factors causing gastro-oesophageal reflux disease still relatively unchanged and a continuing modest relationship between the DeMeester score and the degree of oesophagitis and Barrett's oesophagus, the next question was obviously which components of the refluxate were actually doing the damage.

The classic studies by Safaie-Shirazi had shown that both bile salts and pepsin at the proper pH, breach the mucosal barrier to H+. Subsequent studies, summarised in the section on experimental reflux oesophagitis, indicated wide differences in the effects of various bile acids and pancreatic enzymes and that the effect of pancreatic enzymes could be inhibited by bile acids. At first sight the extensive experimental evidence that on the one hand acid is not the prime mover but on the other hand clinical proof that acid suppression does heal reflux oesophagitis (but not Barrett's oesophagus) suggests that we should be looking at substances which work at a low pH.

Early studies of the components of the refluxate looked at aspirated gastric content, with Gillen only finding significant differences in post-prandial gastric bile acid concentrations between complicated Barrett's oesophagus and 3 other patient groups, including uncomplicated Barrett's oesophagus, reflux oesophagitis and normal controls. Examinations of aspirated oesophageal
refluxate tended to meet with fairly inconclusive results\(^{168-172}\). However, in 1998 Nehra et al.
using an automated sampling device, did manage to establish a hierarchy, similar to that found in
acid reflux, in oesophageal bile acid concentrations between normals, minimal oesophagitis,
erosive oesophagitis and strictures or Barrett’s oesophagus. Mixed reflux of acid and bile acids
was mainly seen in more severe oesophagitis but in each of the 3 patient groups there were two
bile acid refluxers without acid. There was no correlation between total oesophageal bile acid
exposure and acid or alkaline exposure\(^{173}\). Unfortunately, the authors did not present data on the
nature, degree of solubility or conjugation state of the bile acids nor were pancreatic enzymes
determined. The introduction of the Bilitec technique enabled 24-hour oesophageal acid and
bilirubin monitoring. Vaezi et al, using this technique, found that both acid and duodeno-
gastrooesophageal reflux showed a graded increase in severity across the gastro-oesophageal
reflux spectrum. Acid and duodeno-gastroesophageal reflux were found to have occurred
simultaneously in the majority of the reflux episodes\(^{174}\). Banki et al. investigating why Barrett’s
oesophagus was more common in men reviewed the records of 796 patients (462 male, 334
female) evaluated for symptoms of reflux. Physiologic abnormalities based on results of
endoscopic, motility, pH, and Bilitec testing were identified, and factors related to the presence
of Barrett's were determined. Females with reflux symptoms were significantly less likely to
have a positive 24-h pH test, a defective lower oesophageal sphincter, or a hiatal hernia than
males with reflux symptoms. Furthermore females with reflux on the basis of an abnormal 24-hr.
pH test had significantly less oesophageal acid exposure than males with reflux. However, in
spite of the milder abnormalities in females with gastro-oesophageal reflux disease, oesophageal
exposure to refluxed acid and bilirubin was similar in females (n = 50) and males (n = 136) with
Barrett's oesophagus. On multivariable analysis increased oesophageal bilirubin exposure (i.e.
duodeno-gastro-oesophageal reflux) was the only significant factor associated with the presence
of Barrett's oesophagus in both in male and female patients with reflux disease i.e. in severe
oesophageal reflux disease females were no longer protected against Barrett's esophagus, a
conclusion at variance with the far lower percentage of females with Barrett’s oesophagus and
which failed to analyse the higher mean age of the female patients which would be compatible
with a postponement of severe reflux disease and Barrett’s oesophagus\(^{175}\). A study by Stein et al.
not only confirmed an exponential increase in the mean oesophageal bile exposure time for
gastro-oesophageal reflux disease patients without oesophagitis to those with erosive
oesophagitis and benign Barrett's oesophagus, but also found it to be highest in patients with
early carcinoma in Barrett's oesophagus (P <0.01). They triumphantly concluded that
oesophageal bile reflux was not effectively suppressed by medical treatment, this in contrast to
Nissen fundoplication\(^{176}\). This issue was presumably raised because two studies had
demonstrated the, at first sight surprising, finding that acid suppression by means of omeprazole,
while actually increasing the number of, now non-acid, postprandial reflux episodes\(^{177}\), also
resulted in a reduction of duodeno-gastro-oesophageal reflux\(^{178, 179}\). The simple explanation for this reduction is that decreased gastric acid production requires less
pancreatic and duodenal secretions for its neutralisation. However, it is also possible that acid
inhibition increases phase III of the migrating motility complex, resulting in improved gastric
clearance by increased antro-duodenal motility\(^{180}\) which may well be a more important
mechanism for the protection of the duodenum than acid neutralisation\(^{181}\). On the other hand,
patients manifesting persistent oesophagitis under proton pump inhibitors were found to have
more duodeno-gastrooesophageal reflux than those who remained symptomatic in spite of having
healed their oesophagitis\(^{182}\). Although proton pump inhibitors cannot guaranty the control of
duodeno-gastroesophageal reflux\textsuperscript{183}, its reduction may well be the key explanation of how acid inhibition heals reflux oesophagitis.

Unfortunately, while demonstrating that duodeno-gastro-oesophageal reflux plays an essential aetiopathological role in both reflux oesophagitis and Barrett's oesophagus, we are not much better informed than Aylwin\textsuperscript{98} in 1953 about which potentially damaging substances, in what concentrations and state of solubility are present in the oesophagus at any particular moment\textsuperscript{184}. This in contrast to some sophisticated studies looking at the effects of bile acids at the cellular and genetic level\textsuperscript{185,186}.

In the mean time there is a new contender for a leading role in the aetiology of reflux oesophagitis, Barrett's oesophagus and adenocarcinoma of the oesophagus and cardia, cancers which are occurring at the anatomical site where saliva encounters acidic gastric juice and their interaction generates reactive nitrogen species which are potentially mutagenic and carcinogenic\textsuperscript{187}. These encounter sites have also been shown to lie within Barrett's oesophagus\textsuperscript{188}.

An important question, raised by Maley, is whether gastro-oesophageal reflux continues to play a role in the progression from Barrett's oesophagus to adenocarcinoma of the oesophagus. Daily wounding of the Barrett's oesophagus by acid and bile salts may “trigger wound healing responses including mitogens, release from growth inhibition and suppression of apoptosis in the base of the crypts”. This process could explain the “over-expression of a variety of oncogenes, along with the scarcity of oncogenic mutations”. his mechanism is supported by the fact inflammation is commonly observed in Barrett's 'oesophagus and could be responsible for increases in mutagenesis and proliferation. However, in humans this wounding is in practice only seen in reflux oesophagitis\textsuperscript{138}.

This issue of whether Barrett’s oesophagus requires continued mutagenic stimulation by gastro-oesophageal reflux or, once set on the path of oncogenesis, will autonomously follow it to the end, is of obvious practical importance. Moghissi et al. found no effect of anti-reflux surgery in the prevention of adenocarcinoma of the oesophagus\textsuperscript{189}. In 2004 a review by Sonnenberg, citing, amongst others, a meta-analysis by Corey et al.\textsuperscript{190} entitled: “Does a surgical antireflux procedure decrease the incidence of esophageal adenocarcinoma in Barrett's esophagus?” concluded that “Fundoplication does not prevent the occurrence of Barrett's oesophagus nor its progression to oesophageal adenocarcinoma\textsuperscript{191}. There are currently no published systematic studies into the efficacy of proton pump inhibitors in preventing adenocarcinoma of the oesophagus in Barrett’s oesophagus. This question (also including an aspirin arm) is currently the subject of the AspECT trial\textsuperscript{192}. In spite of some optimistic data\textsuperscript{193}, support for the belief in regression of Barrett’s oesophagus under treatment by proton pump inhibitors is not universal\textsuperscript{194,195}.

On the other hand, regression of Barrett’s oesophagus by Roux-en-Y gastric bypass for morbid obesity was reported by Cobey in a single patient\textsuperscript{196}. Csendes et al. reported 78 patients with Barrett’s oesophagus treated by vagotomy and antrectomy in combination with duodenal bile diversion, an operation designed to abolish acid and duodenal reflux into the distal esophagus. After 5 years they observed regression of Barrett’s oesophagus from intestinal to cardiac or fundic mucosa in about 60% of patients\textsuperscript{197}. The same author reported similar regression of intestinal metaplasia to cardiac mucosa in patients 2 years after undergoing gastric by-pass surgery for morbid obesity, again without achieving a total reversal of Barrett’s oesophagus\textsuperscript{198}.

What can be concluded from these studies? The comparison between the disappointing results of both conventional surgical antireflux procedures and proton pump inhibitors with the relative success of the heroic surgical interventions by Cobey\textsuperscript{196} and Csendes\textsuperscript{198}, suggest that only the
complete exclusion of gastro-oesophageal reflux can achieve a certain degree of regression in Barrett’s oesophagus and, hopefully, prevent the development of adenocarcinoma of the oesophagus. Should the latter assumption prove correct, this would demonstrate that continuing gastro-oesophageal reflux is necessary for the progression to malignancy of Barrett’s oesophagus and that this progression can be halted surgically. However, in practice it is questionable whether such mutilating surgery would be acceptable for the prevention of adenocarcinoma of the oesophagus in Barrett’s oesophagus, the risk of which for an individual patient is probably less than 5%.

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41


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Chapter 2

THE INCIDENCE OF ADENOCARCINOMA IN BARRETT’S OESOPHAGUS IN AN INSTITUTIONALISED POPULATION

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ABSTRACT

Objectives. To ascertain the incidence rate of adenocarcinoma in Barrett’s oesophagus (ACO) in a stable population of 28 000 institutionalized intellectually disabled individuals (IDI) in whom the prevalence rate of Barrett’s oesophagus (BO) was previously estimated in a representative sample by 24-hr pH monitoring and endoscopy and in which all cases of ACO were ascertained over a 6 year period. These IDI do not smoke or drink alcohol and are known to have exceptionally high prevalence rates of gastro-oesophageal reflux disease (GORD) and consequently of BO.

Methods. A population comprising 52 038 person years was observed and all cases of ACO were ascertained. On the basis of the representative sample, the percentage of this population with BO was estimated to be 10.8%. ACO incidence rates could then be estimated and compared to those found in a free living population with BO after correction for age and gender differences.

Results. In IDI an incidence rate of ACO of 2.5/1000 person years was found against 6.3/1000 person years in the free living population. However, the age distributions of the IDI and of the free living population were very different, and after correction for this factor there was no significantly lower incidence rate of ACO in the IDI (relative risk : 0.79; p= 0.61).

Conclusions. This is the first reported incidence study of ACO in a stable, well defined population. In contrast to squamous cell carcinoma, our findings suggest only a minor role for smoking and alcohol in the etiology of ACO.
INTRODUCTION

Barrett’s oesophagus (BO) or columnar lined oesophagus and its most important complication, adenocarcinoma in Barrett’s oesophagus (ACO), are the result of longstanding severe gastro-oesophageal reflux disease (GORD)\(^1\,^2\). In adults this metaplastic lining has often undergone specialised intestinal metaplasia entailing a 30-125-fold increased risk of malignancy\(^3\,^4\). BO has been found in between 0.73%\(^5\) and 1%\(^6\) of all patients coming to endoscopy while a prevalence of 1% was established in an autopsy study\(^7\). Rises in the incidence of adenocarcinoma in Barrett’s oesophagus (ACO) ranging between 5- and 8-fold over the last 15-20 years have been reported\(^8\,^9\). The desire to combat this increasingly important gastrointestinal cancer by surveillance of patients with BO has stimulated interest in the incidence rate of ACO in these patients. However, incidence rates of ACO in patients with BO ranging between 1:48\(^10\) and 1:441\(^11\) patient years have been reported. This wide range is likely to have been the result of a number of factors including publication bias\(^12\) and chance. The latter factor is particularly important as these incidence rates were almost all calculated on the basis of the follow up of relatively small cohorts of patients with BO over a period of a few years\(^13\). Ideally, true incidence rates should be established in large scale population-based incidence studies. This would require a stable, geographically defined, population, from which it would be necessary to recruit a random, representative, sample to undergo endoscopy in order to ascertain the prevalence of BO which, in an asymptomatic population, would probably be around 1%\(^7\). Therefore thousands of volunteers would need to be endoscoped in order to obtain meaningful results. In addition, the ascertainment of all cases of ACO over a period of years in a free living population moving in and out of the study area would present considerable difficulties.

However, by combining data from two previously published studies, we were able to construct a population based study of intellectually disabled individuals (IDI) with an IQ <50 in residential care in the Netherlands in whom the prevalence of GORD and consequently of BO, was known to be high\(^14\,^15\). In addition, this population was homogeneous for the non-consumption of tobacco and alcohol while its diet and social conditions were similar. One study ascertained the prevalence rate of BO in a representative sample of IDI drawn from various institutions\(^16\). The second involved an observational study into the incidence of ACO in this stable and well documented population of 28 000 IDI over a period of six years\(^17\).

METHODS

The study population was described elsewhere\(^16\). Briefly, the prevalence rate of BO in IDI was determined during a study in which the prevalence of GORD was established by 24-hour pH monitoring in a random sample of IDI drawn from 7 institutions. This sample was chosen to reflect both the age and gender composition of the inhabitants of these institutions and that of the total population of IDI in the Netherlands in general. 435 IDI were selected, the investigation was successful in 386 individuals (166 females, 220 males). All 24-hour pH measurements were performed by one experienced investigator (C.B.). GORD was diagnosed when the period with a pH < 4 during 24 hrs exceeded 4%, including an upright and a supine episode. Subsequently, all IDI in whom GORD was diagnosed underwent endoscopy by experienced local gastroenterologists. The diagnosis of reflux-esophagitis was made on the basis of the Savary Miller classification.\(^18\).

BO was defined as columnar epithelium at least 3cm above the gastro-oesophageal junction and confirmed histologically.

In a parallel study data on the occurrence of ACO in IDI was collected\(^17\). The medical staffs of 97 institutions were requested to provide clinical data about the residents in their
institutions identified by the Netherlands Cancer Registry between 1989 and 1995 as suffering from histologically confirmed cancer of the oesophagus. All cases of cancer are routinely reported to this registry by the histopathology departments of all hospitals in the Netherlands. 19 of 97 institutions, comprising 987 residents, were unwilling to participate. Residents from the other 78 institutions, comprising 28 000 persons (mean age: 38.7 yrs; 57.1% men, 42.9% women) were entered into a six-year long observational study running from 1-1-1989 to 1-1-1995. ACO was defined as adenocarcinoma of the oesophagus not involving the gastric cardia and preferably, but not necessarily, including BO above the tumour.

The incidence rates of ACO were calculated from the age of 45 as no cases of ACO were found below this age. This was done as follows, after determining the prevalence rate of BO for the over 40-year age-group we estimated the number of person years with BO (i.e. the population at risk) during the follow-up period by multiplying the total number of IDI person years observed for each gender and each age band by the observed prevalence rate of BO. This estimated number of person years with BO was the denominator in the calculation of the age and gender specific incidence rates of ACO.

The whole procedure is summarized in Figure 1.

**Figure 1**

A flow diagram illustrating the various steps involved in this study. In a representative sample of institutionalized intellectually disable individuals (IDI), gastro-oesophageal reflux disease (GORD) was established by 24-hr. pH monitoring, GORD positive IDI were endoscoped and the cases of Barrett’s oesophagus (BO) found served to establish the prevalence of BO in the tested sample and subsequently in the entire IDI population. The over-40 years old BO prevalence rate was used to estimate the number of observed person years (pers.yrs) of IDI with BO. After division by the observed 14 cases of ACO, this produced the ACO incidence rate for all IDI over the age of 40 years. Similar calculations were performed for males and females (not shown).
Subsequently, after correction for age and gender distribution, the incidence rates in IDI and a free living population studied by v.d. Burgh et al were compared by 5-year age cohorts starting from the age of 45. This study was chosen for comparison because it was the most extensive observational study published and showed consistent results at four and nine years follow up. In addition, this population had a similar ethnic background to the IDI.

STATISTICS
The prevalence rates of BO by gender and age group were compared using Fisher’s exact test. The incidence rates of ACO in IDI and the free living population were compared using multivariate analysis (Poisson regression) while taking into account the age band and gender of the subjects in each group.

ETHICAL APPROVAL AND INFORMED CONSENT
The Subcommittee for the Ethics of Human Research of the Academic Hospital Vrije Universiteit in Amsterdam gave her consent for this study. All guardians (legal representatives of the IDI) were asked for informed consent for the anonymous use of medical information for scientific research.

RESULTS
The prevalence rate of GORD, determined by 24-hr. pH monitoring in the random sample was found to be 48%, comprising 44% (82/166) of the females and 56% (104/220) of the males. The prevalence rates of GORD remained constant for both genders over all age groups. Endoscopy in all subjects with GORD revealed reflux-esophagitis in 69%, 68% (56/82) in females and 70% (73/104) in males. BO was found in 22 (11.8%), comprising 36% (8/22) in females and 64% (14/22) in males. The prevalence rate of BO was then calculated for all 386 tested subjects, both GORD positive and negative, assuming that no BO would be found in GORD negative IDI. The prevalence rate of BO was found to be significantly higher in the population over 40 years, 10.8% (14/130), versus 3.1% (8/256) in the under-40 age group, p = 0.004. In the GORD positive over-40 age-group 25.8% (8/31) of the males and 19.4% (6/31) of the females had BO (p = 0.76 n.s.). In the whole tested sample (GORD + and –) 11.3% (8/71) of the males and 10.2% (6/59) of the females had BO (p = 1.0 n.s). Table 1.

Table 1. The prevalence rates of Barrett’s oesophagus (BO) found in the random sample of IDI tested by 24-hr pH monitoring by age group, gender and gastro-oesophageal reflux disease (GORD) status (positive or negative)

<table>
<thead>
<tr>
<th>Age group</th>
<th>GORD Positive (n=186)</th>
<th>GORD Negative (n=200)</th>
<th>ALL TESTED (n=386)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males females total</td>
<td>males females total</td>
<td>males females total</td>
</tr>
<tr>
<td>under</td>
<td>8.2% 3.8% 6.4%</td>
<td>4% 1.9% 3.1%#</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>(6/73) (52/80) (0/76)</td>
<td>(0/131) (6/149) (2/107)</td>
<td></td>
</tr>
<tr>
<td>over</td>
<td>25.8%§ 19.4%§ 21.9%</td>
<td>11.3%* 10.2%* 10.8#</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>(8/31) (6/31) (14/61)</td>
<td>(8/71) (6/59) (14/130)</td>
<td></td>
</tr>
</tbody>
</table>

The prevalence rates of BO are expressed as percentages. The figures between parentheses are: the number of IDI with BO/ number tested in each category. In GORD negative IDI no endoscopies were performed as it was assumed that none had BO. The observed prevalence rate of BO was 10.8%. § difference not significant, p = 0.76, * difference not significant, p = 1.0, # difference significant, p = 0.004.

The over-40 prevalence rate of 10.8% was subsequently used in estimating the number of BO person years (i.e. the population at risk), which in turn was the denominator in calculating ACO incidence rates.
The number of all cancers of the oesophagus and gastric cardia in IDI over the age of 45, observed during 52,038 person years, was 20. Of these two were squamous cell carcinomas, four adenocarcinomas of the gastric cardia and 14 were ACO. After correction for age and gender distribution, the incidence of these first two tumours was in accordance with, but that of ACO considerably in excess of, the expected incidence in the general Dutch population\textsuperscript{19}. The number of person-years observed, the estimated number of BO person-years, the number of cases of ACO and the resulting incidence rates are presented in Table 2.

### Table 2. The number of observed person years for all institutionalized intellectually disabled individuals (IDI) and the estimated number of person years with Barrett’s oesophagus (BO)

<table>
<thead>
<tr>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
<th>Column 5</th>
<th>Column 6</th>
<th>Column 7</th>
<th>Column 8</th>
<th>Column 9</th>
<th>Column 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age bands</td>
<td>All Males</td>
<td>BO pos. x 0.108</td>
<td>All Females</td>
<td>BO pos. x 0.108</td>
<td>All BO pos. M+F x 0.108</td>
<td>Males</td>
<td>Females</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>45-49 years</td>
<td>8156</td>
<td>881</td>
<td>6178</td>
<td>667</td>
<td>14334</td>
<td>1548</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>50-54 years</td>
<td>5432</td>
<td>587</td>
<td>4426</td>
<td>487</td>
<td>9858</td>
<td>1074</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>55-59 years</td>
<td>5247</td>
<td>567</td>
<td>3927</td>
<td>424</td>
<td>9174</td>
<td>991</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>60-64 years</td>
<td>4075</td>
<td>440</td>
<td>2975</td>
<td>321</td>
<td>7050</td>
<td>761</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>65-69 years</td>
<td>2967</td>
<td>320</td>
<td>2265</td>
<td>245</td>
<td>5232</td>
<td>565</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>70-74 years</td>
<td>1641</td>
<td>177</td>
<td>1467</td>
<td>158</td>
<td>3108</td>
<td>335</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>75-79 years</td>
<td>1118</td>
<td>121</td>
<td>850</td>
<td>92</td>
<td>1968</td>
<td>213</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>&gt;79 years</td>
<td>748</td>
<td>82</td>
<td>566</td>
<td>61</td>
<td>1314</td>
<td>143</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>29384</td>
<td>3175</td>
<td>22654</td>
<td>2455</td>
<td>52038</td>
<td>5630</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

Incidence rates: males: 11/3175 = 3.5/1000 person years (column 8/3); females: 3/2455 = 1.2/1000 person years (column 9/5; males + females: 14/5630 = 2.5/1000 person years (column 10/7).

Columns 2 and 4 present the number of person-years by age and gender observed during the six year follow up. Column 6 presents the numbers for both genders combined. Columns 3, 5 and 7 represent the estimated number of observed person years of IDI with BO obtained by multiplying the numbers in columns 2, 4 and 6 by 0.108 (10.8% prevalence rate of BO). Columns 8, 9 and 10 represent the incidence of ACO by age, gender and both genders combined. Incidence rates of ACO were calculated by dividing the totals in columns 8, 9 and 10 by those in columns 3, 5 and 7.

The overall ACO incidence rate for IDI was 2.5/1000 person years. For males this was 3.5/1000 person years and for females 1.2/1000 person years.

The ACO incidence in our free-living population was 6.3/1000 person years. For males this was 9/1000 person years and for females 3.4/1000 person years. (Table 3).

However, after correction for differences regarding the age and gender distributions, there was no significant difference in the incidence rate of ACO between the IDI group and the free living population. The relative risk of ACO in IDI versus our free living population was 0.79 (p = 0.61).

The gender specific ACO incidence rates for both populations showed a male predominance which in IDI just failed to reach significance.
Table 3. A comparison of the estimated observed person-years for intellectually disabled individuals (IDI) and the observed person years for free living patients, by age band and gender, and the incidence of ACO for both populations.

<table>
<thead>
<tr>
<th>Age bands</th>
<th>Males (Person-years)</th>
<th>Females (Person-years)</th>
<th>Males (ACO)</th>
<th>Females (ACO)</th>
<th>Males (Person-years)</th>
<th>Females (Person-years)</th>
<th>Males (ACO)</th>
<th>Females (ACO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-49 years</td>
<td>881 1</td>
<td>667 0</td>
<td>55 0</td>
<td>8 0</td>
<td>321 0</td>
<td>6 0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>50-54 years</td>
<td>587 1</td>
<td>487 0</td>
<td>73 1</td>
<td>6 0</td>
<td>314 0</td>
<td>3 0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>55-59 years</td>
<td>567 1</td>
<td>424 0</td>
<td>88 0</td>
<td>26 1</td>
<td>303 0</td>
<td>2 0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>60-64 years</td>
<td>440 1</td>
<td>321 0</td>
<td>101 0</td>
<td>3 0</td>
<td>293 0</td>
<td>1 0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>65-69 years</td>
<td>320 1</td>
<td>245 0</td>
<td>127 2</td>
<td>62 0</td>
<td>238 0</td>
<td>2 0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>70-74 years</td>
<td>177 3</td>
<td>158 0</td>
<td>120 3</td>
<td>110 0</td>
<td>184 0</td>
<td>3 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75-79 years</td>
<td>121 1</td>
<td>92 2</td>
<td>52 0</td>
<td>146 0</td>
<td>136 0</td>
<td>2 0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;79 years</td>
<td>82 2</td>
<td>61 0</td>
<td>57 0</td>
<td>192 1</td>
<td>105 0</td>
<td>0 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>3 175 11</td>
<td>2 455 3</td>
<td>673 6</td>
<td>589 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incidence rates: 3.5 /1000 person-years (IDI) vs. 1.2 / 1000 person-years (Free living BO cohort) vs. 8.9 / 1000 person-years (Free living population) vs. 3.4 / 1000 person-years (IDI).

**DISCUSSION**

This study was designed to estimate the incidence rate of adenocarcinoma of the oesophagus for individuals with Barrett’s oesophagus (BO) belonging to a unique institutionalized population of Intellectually Disabled Individuals (IDI). The prevalence rate of BO in this population was estimated from a BO prevalence rate previously established in a representative sample of IDI from a number of these institutions. As this prevalence rate was probably at least tenfold higher than in the general population, the incidence of adenocarcinoma of the oesophagus (ACO) found in this population was far higher than would have been found in a general population of the same size and age distribution. In addition, the ascertainment of individuals with BO was unique in not being based on the presence of symptoms leading to endoscopy but on the random selection of individuals to be tested for GORD.

The overall incidence rate of ACO found of 2.5/1000 person years was far lower than both the incidence of 5/1000 person years recently estimated on the basis of 25 BO follow-up studies and the overall ACO incidence rate in our free living population, used for comparison, of 6.3/1000 person years.

This lower incidence rate was, however, both a reflection of the difference in gender distribution and, most importantly, of the life expectancy of IDI, which is far lower than that of the general population. Consequently, their age and gender distribution differed greatly from both that of the population at large and from that of our free living population in having relatively younger individuals. Therefore, adjustment for age and gender distribution was essential for an unbiased comparison. After this correction no significant difference was found between the incidence rate of ACO in our free living population versus the IDI (relative risk 0.79, p=0.61). Therefore, this study, failed to demonstrate different risks of developing ACO for individuals with BO of the same age from both populations. However, in view of its limited size, it was also unable to disprove the existence of some differences in ACO risk in
either directions, i.e. the risk in IDI to be higher or lower than in the free living population. In addition, our findings are a clear example of the fact that a comparison of incidence rates of ACO between various cohorts of patients with BO can only be valid after correction for age and gender differences.

An additional unique feature of this institutionalized population was its homogeneity for the factors non-consumption of tobacco and alcohol, ethnic background, diet and social conditions. The fact that their incidence rate of ACO did not differ significantly from that of our free living population, in which a considerable mortality from smoking related diseases was found supports the modest role of smoking and alcohol consumption in the aetiology of ACO which was recently summarized in a review of case-control studies by Cameron. For smoking he found odds ratios between smokers and controls varying between 1.5 and 3.4, far lower than in squamous cell carcinoma where odds ratios of 10.4 and 16.9 were recorded.

As can be seen in Tables 4 and 5

### Table 4  Smoking and adenocarcinoma: a summary of case-control studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Cases</th>
<th>Smoking: Odds Ratio versus controls (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabbat 1993</td>
<td>Oesophagus. and cardia</td>
<td>122</td>
<td>2.3 (1.4-3.9)</td>
</tr>
<tr>
<td>Brown 1994</td>
<td>Oesophagus. and cardia</td>
<td>174</td>
<td>2.1 (1.2-3.8)</td>
</tr>
<tr>
<td>Vaughan 1995</td>
<td>Oesophagus. and cardia</td>
<td>298</td>
<td>3.4 (1.4-8.0)</td>
</tr>
<tr>
<td>Zhang 1996</td>
<td>Oesophagus. and cardia</td>
<td>95</td>
<td>1.5 (0.7-3.0)</td>
</tr>
<tr>
<td>Gammon 1997</td>
<td>Oesophagus</td>
<td>293</td>
<td>2.2 (1.4-3.3)</td>
</tr>
<tr>
<td></td>
<td>Cardia</td>
<td>261</td>
<td>2.6 (1.7-4.0)</td>
</tr>
<tr>
<td>Lagergren 2000</td>
<td>Oesophagus</td>
<td>189</td>
<td>1.6 (0.9-2.7)</td>
</tr>
<tr>
<td></td>
<td>Cardia</td>
<td>262</td>
<td>4.5 (2.9-7.1)</td>
</tr>
</tbody>
</table>

Tables 4 and 5 data adapted from Cameron with permission of the author.

### Table 5.  Smoking and squamous cell carcinoma: case control studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Cases n.</th>
<th>Smoking: Odds Ratio versus controls (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaughan 1995</td>
<td>Oesophagus</td>
<td>106</td>
<td>16.9 (4.1-69) (&gt;80 pack years)</td>
</tr>
<tr>
<td>Lagergren 2000</td>
<td>Oesophagus</td>
<td>167</td>
<td>10.4 (5.6-19.4) (&gt; 20 cigarettes &gt;35 yrs)</td>
</tr>
</tbody>
</table>

On the influence of alcohol, Cameron found only a modest correlation with heavy alcohol intake in 3 of 6 case-control studies, but no correlation in the other 3 which included the two largest. Although not reaching statistical significance, we found a higher incidence rate of ACO in male IDI compared to that of females of about 3 to 1, which was actually lower than published sex ratios in the incidence rates of ACO in the general population ranging between 6 and 8 to 1.

In theory this sex ratio could merely reflect the lower prevalence rate of BO in females but no gender specific differences in the prevalence rates of BO of this magnitude have as yet been published and the cause of this excess male incidence is as yet unexplained.
However, in IDI the excess male ACO incidence ratio cannot have been caused by differing lifestyles, including smoking and would appear to reflect some inherent protection against ACO by female gender.

Obviously, the reliability of the incidence of ACO calculated for the IDI population hinged on the accuracy with which the prevalence of BO was ascertained in the sample population. This prevalence rate was determined by means of a random sample of IDI drawn from 7 institutions, which was subsequently shown to be representative for the total population of IDI in the Netherlands. The diagnosis of GORD by 24-hour pH measurement is a well established technique. The separation between normal and pathological reflux in the sample of IDI was extremely clear-cut with only 4 cases in the intermediate zone with a pH < 4 between 4.5-6% of the measured time. Also the prevalence rate of GORD remained constant over the various age-groups. There was a significant rise in the prevalence of BO around age 40, similar to that found in other studies. In addition smaller studies found similar prevalence rates of BO in IDI. Finally, the diagnosis of BO was established by 5 experienced endoscopists and confirmed by histology.

IDI without GORD did not undergo endoscopy because it was assumed that BO would not occur in the absence of GORD. However, two studies suggested that this combination could occur and the authors kindly communicated their detailed data. They found 6 of 51 and 3 of 16 cases of BO respectively (a total of 9/66) with 24-hour pH measurements within the normal range. As there is no published data on the prevalence of BO in an unselected population without GORD, it was impossible to calculate a statistically reliable figure for the prevalence of BO in IDI without GORD. However, on the basis of these 9/66 cases of BO without GORD, it was estimated that 3 such cases could have been missed in the prevalence study through false negative 24-hour pH measurements. The addition of these 3 cases to the original 14 would have increased the BO prevalence rate to 13% instead of 10.8%. However, recalculations with this higher prevalence rate resulted in ACO incidence rates for IDI which again did not significantly differ from that found in our free living population, with a relative risk of 0.65, (p=0.37).

It could be argued that IDI, by the nature of their condition, cannot be considered a suitable model for the effects of GORD in the intellectually normal population. However, besides their I.Q., their only fundamental difference from the general population, lies in their abnormally high prevalence of GORD for which no clear explanation has yet been found but is already present from childhood, the most important factor appearing to be an IQ < 35.

In addition, the prevalence of BO in IDI with GORD was not higher than that found in studies of patients with GORD of normal intelligence. The ascertainment of ACO in the population of IDI is likely to have been accurate.

Oesophageal cancer invariably becomes symptomatic. The fact that some IDI with ACO would have been asymptomatic at the end of the observation period is offset by the fact that a similar number would have been asymptomatic at the start of observation period. In addition, the incidence of squamous cell carcinomas and carcinomas of the gastric cardia was in accordance with the age related incidence in the general population.

In conclusion, this is the first observational study into the incidence rate of ACO in a stable, well observed and defined population in which the prevalence rate of BO was first established by a randomly selected representative sample. As this population does not consume alcohol or tobacco this incidence rate, corrected for age and gender, should be considered as the minimum for observational studies. That this incidence rate did not differ significantly from
that found in a free living population supports the case control studies indicating that the role of alcohol and tobacco in the aetiology of ACO is far smaller than in squamous cell carcinoma of the oesophagus. In addition, our data do not support the theory that the lower incidence rate of ACO in females mainly results from differences in lifestyles such as smoking or alcohol consumption.

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Chapter 3

THE AGE AND SEX DISTRIBUTION OF THE PREVALENCE OF BARRETT’S OESOPHAGUS FOUND IN A PRIMARY REFERRAL ENDOSCOPY CENTRE.

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ABSTRACT

Background: Both the demographics underlying the sex ratio in the prevalence of Barrett’s oesophagus (BO) and the status of BO without intestinal metaplasia (IM) is unclear.

Aims: To establish the demographics of histologically proven BO, IM positive and IM negative, over a 15 year period from a primary referral, endoscopy unit.

Patients and methods: For all BO patients aged 20-89 identified between 1982-96, IM positive or IM negative, prevalence rates were calculated per 100 first endoscopies.

Results. 492 cases of BO, 320 (248 IM positive) in males, 172 (127 IM positive) in females were identified in 21,899 first endoscopies (10,939 males, 10,960 females). Between ages 20-59 in males and 20-79 in females, IM positive, IM negative and all BO prevalence rates rose by ±7.36% for each additional year of age (p=0.92) with however, a 20 year age shift between the sexes, resulting in an male: female O.R. 4.15 95%CI 2.99-5.77. A declining rate of increase in over 59 males resulted in an overall male: female O.R. 2.14, 95%C.I. 1.77-2.58. Over the age of 79, BO prevalence rates/100 first endoscopies fell from a maximum of 5.1 in males and 3.65 in females to 3.38 and 2.53 respectively.

Conclusion: The 4:1 sex ratio and 20 year age shift between males and females in the prevalence of BO, both IM positive and IM negative, found in younger age groups, was the main cause of the overall BO 2:1 sex ratio. The very similar demographics of IM negative and IM positive BO suggest they may be 2 consecutive stages in the same metaplastic process.
INTRODUCTION

Over the last 50 years Barrett’s oesophagus (BO) has evolved from a medical oddity to a pre-malignant condition thought to lie at the root of most, if not all, cases of adenocarcinoma of the oesophagus (ACO) which constitutes the gastrointestinal malignancy with the fastest growing incidence in the industrialised world. Considerable effort is currently directed towards secondary prevention by means of endoscopic surveillance of the about 5% of the individuals identified with BO. Designing effective preventive strategies for patients with BO requires a greater knowledge of the epidemiology of BO. The usual methods of ascertaining BO, endoscopy with biopsy and the less usual, autopsy, are neither acceptable nor feasible for large scale population surveys. In addition, a large proportion of the 95% unidentified BO population is asymptomatic, probably because of their relatively insensitivity to reflux symptoms. Consequently, only random surveys would be able to ascertain the true prevalence of BO in the general population. Attempts in patients undergoing colonoscopy have produced conflicting results. A 7% prevalence of long segment BO was found in a group of 110 asymptomatic, predominantly male, veterans with a mean age of 61 years. In contrast, only 1.2% long segment BO was reported from a group of 961 patients, mean age 59 years, including symptomatic patients, 40% women and 20% blacks. Therefore, large scale epidemiological data can, as yet, only be derived from findings in patients who have undergone endoscopy but who, for reason of selection bias, cannot be representative for the whole BO population. Consequently no conclusions about the BO prevalence within the general population can be drawn from such studies. However, the 95% ‘silent majority’ with BO who ultimately present with ACO was found to display a male predominance and an age specific incidence rise similar to that found in BO prevalence. Therefore, in the absence of better data, the age and sex specific BO prevalence rates found in large series of endoscoped patients from primary referral centres may serve to provide an impression of the relative BO prevalence rates within the population of their catchment areas and help in understanding BO sex ratios.

A previously published study on the occurrence of BO provided the data base for the present study. It came from a single endoscopy unit in a community hospital serving an area of over 400,000 inhabitants over the age of 20 and to which patients were directly referred by general practitioners, thus avoiding the accumulation of selected cases often found in referral centres. These existing data were reanalyzed by calculating the prevalence of BO, including cases with (IM positive) and without (IM negative) intestinal metaplasia (IM), per 100 first endoscopies for both sexes by 10-year age bands over the 3 consecutive 5-year periods. These results were then analyzed statistically in order to establish the constancy in pattern of prevalence rates for all three categories over each of the three 5-year periods, to search for the origins of the sex ratios in BO prevalence rates and investigate demographic differences between IM positive and IM negative BO.

METHODS

Endoscopies at the Wexham Park Hospital endoscopy unit were performed by a team of endoscopists under supervision of a senior endoscopist with a special interest in BO. All original upper gastrointestinal endoscopy paper report forms from this unit for the period January 1977 to December 1996 were searched manually (From 1990 information was kept on an
electronic database) looking for description of features attributable to BO or mentioning BO in the diagnosis. Histology reports were attached to the original endoscopy reports. A diagnosis of BO was made when the squamo-columnar junction was seen to be proximal to the upper limit of the gastric folds, whatever the length of lesion. Only the 80% of cases where there was histological confirmation from biopsies taken from the correct site were included in this series. BO was diagnosed when the biopsies showed columnar type mucosa with either gastric-type mucosa or intestinal metaplasia (IM). No specialist staining was performed for IM but this was recognized by the presence of goblet cells on H&E stained sections. If seen, IM was automatically mentioned in the histopathology report. Gastric-type mucosa was included in our analysis as it was thought to be a possible precursor of IM22.

Results from the period 1977-1981 period were discarded as the number of cases of BO identified was far smaller than in the subsequent 5-year periods. In addition, results from the age bands 0-19 and 90+ were also discarded as both the number of cases of BO and the number of endoscopies were too small to provide meaningful data. In order to avoid overestimation of the number of endoscopies in calculating prevalence rates, it was essential that the numerator should only consist of first endoscopies. This was established by entering all patient identifiers on a computer database and removal of multiple records for the same patient. The prevalence rates of BO (IM positive or IM negative) per 100 first endoscopies could then be calculated from the number of cases of BO (IM positive or IM negative) by sex, 10-year age bands and 5-year periods.

STATISTICS.
Logistic regression analysis of the prevalence results was applied in three steps. The first was to identify possible differences by sex for the age specific BO (IM positive or IM negative) prevalence rates between the three 5-year periods by the interaction of age and sex plus the three 5-year periods (age and period as categorical variables). The second looked for differences by sex in the patterns of the age specific BO (IM positive or IM negative) prevalence rates by the addition of the interaction of the age and 5-year period.

In the third step the exponential segments (for males from ages 20-59, for females from ages 20-79) of the age specific rise in the prevalence rates of BO (IM positive or IM negative) for both sexes, which appeared to display specific patterns, were further analyzed by estimating their rates of increase for each additional year of age by logistic regression. This resulted in sigmoid curves. To fit these curves age was now used as a continuous predictor, with the mean ages per age band as values. This allowed the estimation of BO (IM positive or IM negative) prevalence rates per 100 first endoscopies for every possible age within the age ranges over which logistic regression of the rates of increase was consistent with a simple sigmoid curve.

RESULTS
The percentage distribution of the population over the age of 20 of the catchment area for the endoscopy unit and a similar percentage breakdown of the endoscoped population by 10-year age bands and sex are displayed in figure 1. There were considerable differences between these two age distributions. In addition, it can be seen that there were relatively more males endoscoped between the ages of 20-40 and relatively more females over the age of 70. However, as the BO prevalence rates were calculated per 100 first endoscopies, these were not influenced by these discrepancies.
Figure 1. A comparison between the age and sex distribution of the population of the catchment area in 1991 and that of the patients undergoing a first endoscopy at Wexham Park Hospital between 1982 and 1996.

The number of cases of BO (IM positive or IM negative) identified, tabulated by age and sex, together with the number of first endoscopies and the resulting BO (IM positive or IM negative) prevalence rates/100 first endoscopies for both the three 5-year periods and the overall 15-year periods, are shown in Tables 1 and 2 and are graphically presented in Figures 2, 3 and 4. In males the number of first endoscopies was 11 195, identifying 320 cases of BO, 248 IM positive and 72 IM negative, in females these figures were 11 211 and 172, 127 IM positive and 45 IM negative respectively. The mean age at diagnosis of BO was about 62 for males and 72 for females. In both sexes, the levels of the BO prevalence rates for the periods 1982-1986 and 1987-1991 were similar. However, those for 1992-96 were 50% higher (p<0.001) than found in the two previous 5-yr periods. In both sexes the patterns of the age specific IM positive, IM negative, and all BO prevalence rates, as shown in Figures 1 and 2, did not differ significantly for the three 5-yr periods and the total 15-yr period (p = 0.34 for all males, p = 0.55 for IM positive males and p = 0.13 for all females, p = 0.07 for IM positive females.)

In males, the age specific BO prevalence rates for 1982-96 rose rapidly between the ages of 20 and 59 to 3.83/100 first endoscopies (3.1 IM positive, 0.73 IM negative), after which the rate of increase fell sharply. The male BO prevalence rates eventually reached a maximum of 5.1/100 first endoscopies (3.96 IM positive, 1.14 IM negative) in the 70-79 age band. In females, the age specific BO prevalence rates for 1982-96 were initially far lower, only reaching the 1.0/100 first endoscopies level in the 55-59 age band. They eventually attained a maximum BO prevalence of 3.65/100 first endoscopies (2.7 IM positive, 0.95 IM negative) in the 70-79 age band. The maximum BO prevalence for both sexes combined in the 70-79 age band was 4.29/100 first endoscopies (3.26 IM positive, 1.03 IM negative). Above the age of 59 for males and the age of 79 for females, the rates of increase in the BO prevalence rates were no longer consistent with a simple sigmoid curve.
Table 1. The Numbers and Prevalence rates/ 100 First Endoscopies of IM Positive, IM Negative and all Cases of Barrett’s Oesophagus, by 10-year Age Bands for the Three 5-year Periods and for the Total 15-year Period.

<table>
<thead>
<tr>
<th></th>
<th>Age bands</th>
<th>Males</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70-79</th>
<th>80-89</th>
<th>Totals</th>
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<td>5-year periods</td>
<td>Endoscopies n.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>1982-86 BO IM positive.</td>
<td></td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>9</td>
<td>11</td>
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<td>3</td>
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<td></td>
<td>0.2</td>
<td>1.57</td>
<td>1.55</td>
<td>1.96</td>
<td>3.67</td>
<td>2.65</td>
<td>1.49</td>
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<td></td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>21</td>
<td></td>
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<td>0.42</td>
<td>0.20</td>
<td>0.69</td>
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<td>0.98</td>
<td>0.88</td>
<td>0.67</td>
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<tr>
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<td></td>
<td>3</td>
<td>9</td>
<td>13</td>
<td>21</td>
<td>18</td>
<td>4</td>
<td>68</td>
<td></td>
<td></td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0</td>
<td>0.62</td>
<td>1.77</td>
<td>2.24</td>
<td>3.2</td>
<td>4.65</td>
<td>3.53</td>
<td>2.16</td>
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<td>1987-91 Endoscopies n.</td>
<td></td>
<td>405</td>
<td>480</td>
<td>555</td>
<td>595</td>
<td>675</td>
<td>511</td>
<td>182</td>
<td>3487</td>
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<tr>
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<td></td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>13</td>
<td>23</td>
<td>12</td>
<td>3</td>
<td>60</td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0.83</td>
<td>0.9</td>
<td>2.18</td>
<td>3.4</td>
<td>2.35</td>
<td>1.65</td>
<td>1.72</td>
<td></td>
<td></td>
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<tr>
<td>BO IM negative</td>
<td></td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td>25</td>
<td></td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0.49</td>
<td>0.42</td>
<td>0.18</td>
<td>0.68</td>
<td>0.75</td>
<td>1.95</td>
<td>0.55</td>
<td>0.72</td>
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<td>BO total n.</td>
<td></td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>17</td>
<td>28</td>
<td>22</td>
<td>4</td>
<td>85</td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0.49</td>
<td>1.25</td>
<td>1.08</td>
<td>2.86</td>
<td>4.15</td>
<td>4.3</td>
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<td></td>
<td>441</td>
<td>691</td>
<td>744</td>
<td>758</td>
<td>838</td>
<td>695</td>
<td>297</td>
<td>4555</td>
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<td>7</td>
<td>16</td>
<td>38</td>
<td>33</td>
<td>37</td>
<td>10</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td></td>
<td>0</td>
<td>1</td>
<td>2.15</td>
<td>5</td>
<td>3.94</td>
<td>5.32</td>
<td>3.37</td>
<td>3.1</td>
<td></td>
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<tr>
<td>BO IM negative</td>
<td></td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>26</td>
<td></td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0</td>
<td>0.3</td>
<td>0.67</td>
<td>0.8</td>
<td>0.71</td>
<td>0.68</td>
<td>0.67</td>
<td>0.56</td>
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<tr>
<td>All BO n.</td>
<td></td>
<td>0</td>
<td>9</td>
<td>21</td>
<td>44</td>
<td>39</td>
<td>42</td>
<td>12</td>
<td>167</td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0.49</td>
<td>1.3</td>
<td>2.82</td>
<td>5.8</td>
<td>4.65</td>
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<td>6.04</td>
<td>3.66</td>
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<tr>
<td>15-yr period</td>
<td>Endoscopies n.</td>
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<td></td>
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<tr>
<td>1982-1996 BO IM positive.</td>
<td></td>
<td>1262</td>
<td>1655</td>
<td>1807</td>
<td>1933</td>
<td>2075</td>
<td>1615</td>
<td>592</td>
<td>11195</td>
<td></td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0</td>
<td>12</td>
<td>29</td>
<td>60</td>
<td>67</td>
<td>64</td>
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<td>BO IM negative</td>
<td></td>
<td>0</td>
<td>0.73</td>
<td>1.6</td>
<td>3.1</td>
<td>3.23</td>
<td>3.96</td>
<td>2.7</td>
<td>2.21</td>
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<tr>
<td>Prevalence</td>
<td></td>
<td>0.16</td>
<td>0.36</td>
<td>0.4</td>
<td>0.73</td>
<td>1.01</td>
<td>1.14</td>
<td>0.68</td>
<td>0.65</td>
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<td>All BO n.</td>
<td></td>
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<td>18</td>
<td>36</td>
<td>74</td>
<td>88</td>
<td>82</td>
<td>20</td>
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<td>Prevalence</td>
<td></td>
<td>0.16</td>
<td>1.09</td>
<td>2</td>
<td>3.83</td>
<td>4.24</td>
<td>5.1</td>
<td>3.38</td>
<td>2.86</td>
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</table>
Subsequently, in the 80-89 age band the BO prevalence rates declined steeply in both sexes to 3.38/100 first endoscopies (2.7 IM positive, 0.68 IM negative) in males and 2.53/100 first endoscopies (1.81 IM positive, 0.72 IM negative) in females. No significant differences between the age specific rates of increase in the prevalence rates of IM positive, IM negative and all BO were found, males p=0.23, females p=0.86. (Tables 1 and 2, Figure 4).

Logistic regression applied to the BO prevalence rates for males between the ages of 20-59 and for females between the ages of 20-79, demonstrated that for each additional year of age, the BO prevalence rates in both sexes increased by similar percentages: 7.59% for IM positive, 6.51% for IM negative and 7.36% for all BO, p=0.92. (Figure 5) For these two limited age ranges a male: female O.R. of 4.15, 95% C.I. 2.99- 5.77 was found.
Because the overall age specific BO prevalence curves were non-linear, the overall age shift between males and females could not be calculated. However, as shown above, for males between the ages of 20 and 59 and females between the ages of 20 and 79, this age shift was 20 years. For the whole age range between 20 and 89, after correction for age and period, logistic regression demonstrated the BO prevalence in males to be double that in females, O.R.2.14, 95% C.I.1.77-2.85. However, because of the age shift mentioned above, this O.R. was strongly influenced by the age distribution of patients examined i.e. a different age distribution would have resulted in a different outcome.

**Figure 2.** The male BO prevalence rates/100 first endoscopies for the three 5-year periods and all BO and IM positives for the whole 15-year period. There were no significant differences between the patterns found for each of the three 5-year periods and both 15-year periods, p=0.34 for all BO, p=0.55 for IM positives.
Figure 3. The female BO prevalence rates/100 first endoscopies for the three 5-year periods and all BO and the IM positives for the whole 15-year period. There were no significant differences between the patterns found for each of the three 5-year periods and both 15-year periods, $p=0.13$ for all BO, $p=0.07$ for IM positives.
DISCUSSION

This study analyzed the demographics of a population of almost 500 subjects with Barrett’s oesophagus (BO) and by the inclusion of all cases of columnar lined oesophagus, made possible a demographic comparison of subjects with and without intestinal metaplasia (IM). In addition, it demonstrated the origin of the overall male: female ratio of 2:1 in the prevalence rates of BO. We did not attempt to extrapolate BO prevalence rates from our patients undergoing endoscopy to BO prevalence rates for the catchment population. A valid extrapolation would require two conditions. The first was a similar age and sex distribution between the catchment population and the patients undergoing endoscopy. Figure 1 shows that this was not the case. More importantly, the patients should be selected at random, a condition obviously never met in clinical medicine. In patients with symptoms of reflux, the prevalence of BO is known to be far in excess of that found in those with other symptoms\textsuperscript{17, 23, 24}. On the other hand, between 35% and 48% of the patients presenting with adenocarcinoma of the oesophagus (ACO) and therefore with BO, had no history of reflux symptoms\textsuperscript{2, 8, 9, 25, 26}. Therefore, in clinical endoscopy there are factors leading to both over- and to under diagnosis of BO and their relative proportions are unknown. However, a large proportion of the BO population is likely to be missed by clinical endoscopy\textsuperscript{27}. Both the size of our sample of 22 400 (5.5%) of the 404 000 inhabitants over the age of 20 in our catchment area undergoing a first endoscopy and the consistent BO prevalence patterns over the three 5-year periods (Figures 2 and 3), encouraged us to suppose that the patterns of the age and sex specific prevalence rates and the sex-ratios found in this study provided the best available estimate of these parameters within the BO population of this particular district. For both sexes the age specific BO prevalence rates/100 endoscopies increased without any significant differences between IM positive, IM negative, and all BO individuals (Figure 4).
In addition, for males between the ages of 20 to 59 and for females between the ages of 20-79, the prevalence rates for IM positive, IM negative and all BO individuals increased by practically identical percentages (7.59%, 6.51% and 7.36% respectively, p=0.92) for each additional year of age. (Figure 5) Over this limited age range, the male: female O.R. was in excess of 4:1 and an age shift was found between the parallel incidence rate curves of 20 years. Conceptually, the female curve could be considered to have started from the same value but 20 years later than the male curve. In males over the age of 59 the rate of increase in the BO prevalence rates for each additional year of age suddenly declined, causing the logistic regression model for males to be terminated at age 59.

Had the 7.36% rate of increase in males been maintained after the age of 59, this would have resulted in over 400 cases of BO between the ages of 59 and 79, instead of the 170 actually observed. The sudden onset of a competing cause of death, specifically affecting over 50% of the males with BO in this age group is unlikely. The absence of a similar decline in females below the age of 79 would also appear to contradict, although not rule out, a birth cohort effect. This effect describes a continuous rise in the BO prevalence rates in consecutive birth cohorts, resulting in relatively lower BO prevalence rates in the elderly.

Whatever the cause, the ‘loss’ of over 50% of the potential males with BO over age 59, resulted in the final overall (ages 20-89 for both sexes) male: female O.R. of 2.14. This O.R. was mainly the result of the 20-year age shift between male and female age specific prevalence rates. Male: female ratios in the prevalence of BO ranging between 2:1 and 4:1:1 were found in 3 previous studies. Both the GOSPE and our study (Figure 4) found a slower age specific rise in the female BO prevalence rates under the age of 60. In contrast, a Mayo Clinic study found a parallel rise in the age specific BO prevalence rates for both sexes and here an age shift was obviously absent. The reason for this difference between European and US female age specific BO prevalence patterns is not apparent.

The maximum prevalence rates for both sexes combined in both the GOSPE and the Mayo Clinic study were about 1%. This figure recurred in a later update of the Mayo Clinic data for the total of 12 097 residents of Olmsted County undergoing endoscopy in the 33 years between 1965-97. Our study, derived from 22 400 first endoscopies in 15 years, found a maximum BO prevalence for both sexes combined of 4.29%. This difference may indicate both true regional differences in prevalence rates or in referral patterns.

As in the two earlier studies we did observe a steep fall in the BO prevalence rates after the age of 79 in both sexes. A recent study from the UK National Barrett’s Oesophagus Registry, comprising 5717 patients with BO, found a mean age at diagnosis of BO of 61.4±14.5 for males and 67.5±13.9 for females. Although these were not prevalence rates and may have been skewed by a higher endoscopy rate in older people, the clear difference between the mean ages for both sexes do support the existence of an age shift. In addition, the mean age at diagnosis of ACO in this study was 64.7±8.2 for males and 74.0±8.5 for females, again suggesting a later onset of BO in females.

The 4.15:1 sex ratio found in the young and middle-aged groups could help to explain the 6-8 fold higher incidence rates of ACO in males. As the period between developing BO and symptomatic ACO probably spans decades, most victims of ACO are likely to have acquired BO before the age of 60. In addition, with females developing BO at a higher age than males, many would not survive long enough to progress to symptomatic ACO.
The age shift of 20 years in the onset of BO in females found in our study resulted in a low BO prevalence in females during their reproductive years, possibly by female hormones affording a certain degree of protection against the development of BO. A recent study of over 1800 patients with severe reflux esophagitis (grades C and D) and an earlier population based study both found a 2:1 male: female ratio in the prevalence of oesophagitis, again suggesting a certain degree of protection against reflux esophagitis enjoyed by females.

There is evidence that the age specific increase in the severity of reflux esophagitis is caused by a diminished protective saliva response to reflux with increasing age, however, there are no data on female hormonal effects on saliva or oesophageal mucosal secretions.

The, statistically significant, increased BO prevalence rates found during the last 5-year period cannot be interpreted as certain evidence of a rising prevalence of BO. They could have resulted from of a greater awareness of the endoscopists to the presence of BO, the inclusion of more patients with short segment BO or a change in referral patterns. However, the cohort effect mentioned earlier could well have been partly responsible.

Our inclusion of gastric-type metaplasia in the diagnosis of BO was contrary to current fashion which demands the presence of specialized intestinal metaplasia (IM), including goblet cells, as ACO was found to develop exclusively in this type of metaplasia and the finding gastric type metaplasia was thought to be the result of too distal biopsies. However, it is inconceivable that a series of biopsies accidentally located outside the oesophagus could have resulted in IM negative demographics which were statistically indistinguishable from those of the IM positive category found in this study. It is, however, quite possible that biopsies

![Logistic regression, Males and Females](image-url)
were taken from IM negative areas adjacent to IM positive areas. In a study describing the relationship between the length of columnar epithelium and the presence of IM, all cases of long segment columnar epithelium the oesophagus were also found to contain cardiac type mucosa, while, in spite of extensive biopsies, no IM was found in 15/7146. Consequently, there were probably a number of false IM negative biopsies but, in view of the demographic similarity between IM positive and IM negative, these did not influence the all BO results. We propose that IM negative gastric-type metaplasia in the oesophagus may be the first stage after squamous to columnar cell metaplasia. This metaplasia was recently demonstrated in vitro and can occur rapidly in vivo. Subsequent stages, comprising IM positive metaplasia, low-grade and high grade dysplasia, finally result in ACO. This concept was confirmed by recent data documenting the occurrence of 5 cases of ACO during the follow-up of 137 IM negative subjects. There would then seem to be no reason for withholding the diagnosis of BO from patients with IM negative gastric-type metaplasia found in the oesophagus and, consequently, depriving them of the possible benefits of endoscopic surveillance.

In conclusion, this study of the prevalence of histologically confirmed BO in over 22 000 patients undergoing endoscopy in a primary referral centre, demonstrated that the observed overall 2:1 sex ratio in the BO prevalence rates, predominantly resulted from a 20 year age shift between the parallel age specific BO prevalence curves, for males between the ages of 20-59 and for females between the ages of 20-79. During this period the BO sex ratio was in excess of 4:1. This age shift resulted in relatively low age specific BO prevalence rates in females during their reproductive years. The practically indistinguishable demographics of IM positive and IM negative subjects suggest that they represent consecutive stages in the same metaplastic continuum.

REFERENCES
THE INCIDENCE OF ADENOCARCINOMA AND SQUAMOUS CELL CARCINOMA OF THE OESOPHAGUS; BARRETT’S OESOPHAGUS MAKES A DIFFERENCE.

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ABSTRACT.

Background: Adenocarcinoma limited to the oesophagus (ACO) arises in Barrett’s oesophagus (BO). The incidence of ACO is therefore restricted to this BO sub-population, whose size is unknown and which is for 95% unidentified.

Aims: To determine the age and gender specific incidence rates of ACO, limited to the BO sub-population, within a defined geographical area and to compare them with those of squamous cell carcinoma of the oesophagus (SCC), which can affect the entire population.

Methods: The age and gender specific incidence rates for ACO and adenocarcinoma of the cardia (AGC) were calculated after an expert panel classified 87% of all cases of adenocarcinoma of the oesophagus reported to the Danish Cancer Registry over a six year period as ACO or AGC.

Results: The age specific incidence rates of ACO for males rose from $0.09/10^5$ (30-34 yr) to $14.14/10^5$ (80-84 yr), falling to $7.2/10^5$ (85+ yr), for females from $0.19/10^5$ (45-49 yr) to $2.79/10^5$ (80-84 yr), falling to $2.43/10^5$ (85+ yr) and yielding a gender ratio of 5.9:1; AGC demonstrated a similar pattern and a gender ratio of 4.26:1. However, the incidence rates of SCC continued rising after age-80, with a gender ratio of 2.5:1.

Conclusions: The continuing rise in the SCC incidence rates in the elderly demonstrated that the unexpected decline and fall in the incidence rates of ACO over age-80 did not result from under diagnosis but were most probably caused by a declining prevalence rate of BO, restricting the elderly BO sub-population at risk of developing ACO.
INTRODUCTION
The rate of increase in the incidence of adenocarcinoma of the oesophagus and the gastric cardia is currently thought to exceed that of all other cancers in many western industrialized countries. Gastro-oesophageal reflux and specifically its pre-malignant complication, Barrett’s oesophagus (BO), are the conditions which are associated with both adenocarcinoma limited to the oesophagus (ACO) and, to a lesser extent, with adenocarcinoma of the gastric cardia (AGC).

The incidence rates of cancers in the general population are influenced by a large number of environmental and genetic factors. The population at risk can generally be defined by criteria such as age, gender, lifestyle or occupation. However, both the size of the BO sub-population and the individuals involved are largely unknown. In common with other tumours, the size and the age and gender composition of the BO sub-population at risk must influence the incidence of ACO. However, individuals with BO can only be identified by clinical endoscopy for symptoms, which inevitably introduces selection bias. While BO is over represented in patients with reflux symptoms, these are known to be absent in between 35% and 48% of BO patients presenting with ACO. In addition, 15-20% of the general population regularly report reflux symptoms. Consequently, the ‘silent majority’ of BO patients without clinically significant reflux symptoms leading to endoscopy is even higher. This was reflected in a systematic review of patients coming to surgery for ACO in which only about 5% had a previous diagnosis of BO. This suggests that the extent and composition of the majority of this unidentified BO population can currently only be gauged indirectly through the incidence rates of ACO in the population at large.

In contrast to ACO, which only affects the small segment of the population with BO, the entire population is at risk of developing squamous cell carcinoma of the oesophagus (SCC). A comparison between the age and gender specific patterns of incidence rates of these two tumours within the same organ, with similar symptoms and methods of diagnosis, might be able to provide some hints about the composition of the BO sub-population. Such a comparison required population based incidence data for both ACO and SCC. The latter were readily available. However, population based studies of the age and gender specific incidence rates of ACO were limited to a single publication. This may well be because performance of such studies is handicapped by the lack of reliable data on the true number of cases of ACO, as it is hard to distinguish between ACO and AGC and cancer registries have to rely on the diagnostic accuracy of the reporting clinicians. AGC is a complicated tumour and in the absence of clear genetic or genomic criteria, differentiation between ACO and AGC will continue to depend on complex anatomical criteria. Another potential weakness of population based studies is under reporting in the elderly as a result of medical under diagnosis.

The present study set out to establish basic epidemiological data for the three cancers of the oesophagus and gastro-oesophageal junction, i.e. age and gender specific incidence rates for ACO, AGC and SCC, within a large, geographically defined population. It utilized data from a previous Danish study ‘Adenocarcinoma of the oesophagus and Barrett’s oesophagus’, which originally focused on both the eight-fold increase in the incidence of adenocarcinoma of the oesophagus between 1970-91 and the clinical aspects of the ACO patients. An additional feature provided essential data for the current study. It consisted of the results of a single panel of experts analyzing the clinical data on all adenocarcinomas of the oesophagus reported to the Danish Cancer Registry over a six-year period.
Their differentiation between ACO and ACG should ensure more incidence study we added both the age and gender of all patients to the original data base and the SCC incidence data from the Danish Cancer Registry in order to enable age and gender specific incidence rates for ACO, AGC and SCC to be calculated.
We were then able to compare the incidence rates of ACO and SCC within the same population and over the same 6-year period.

METHODS
These have in part been reported in detail in a previous publication. Briefly, the study population comprised all cases of cancer of the oesophagus reported to the Danish Cancer Registry over the 6-year period between 1987-92. Tumours were classified according to a modified version of the International Classification of Diseases (ICD-7), expanded to include information about histology and tumour behaviour. The Danish Cancer Registry is population based, nation-wide and regarded as almost complete. The incidence data for squamous cell carcinomas of the oesophagus (SCC) (ICD-7 code 150.0) were taken directly from this register. For the cases of adenocarcinoma of the oesophagus (ICD-7 code 150.1) the original medical records were retrieved for review and, after the exclusion of 15 misclassified cases, there remained 580 cases of histologically confirmed adenocarcinoma of the oesophagus and gastric cardia.
Subsequently endoscopic, radiological, surgical and autopsy reports were used to determine the location of the tumour in relation to the gastro-oesophageal junction. Tumours located entirely within the oesophagus were classified as ACO, tumours within the oesophagus but extending across the gastro-oesophageal junction as AGC.
All classifications were performed by a panel of 4 medical gastroenterologists.
After adding the age and gender of all cases of ACO, AGC and SCC to the original data base mean one-year incidence rates were calculated from the number of cases of ACO, AGC and SCC, reported during the six year period, by age band and gender. Population data were derived from Danish population statistics for 1990. The following formula was used:

\[
\text{incidence rate/100 000} = \frac{100 000 \times n_{ACO}}{n_{population} \times 6}
\]

The relations between the age and gender specific incidence rates for ACO and SCC were investigated by log linear regression which fitted curve s for males and females. The standard errors resulting from the regression output allowed 95% confidence intervals to be constructed around these gender specific incidence ratios. Quadratic curves were fitted to the observed incidence rates for ACO and SCC by 5-year age band and tested for age-specific differences in shape by the likelihood ratio test. This last analysis was repeated in a data subset limited to the over 60-year ACO and SCC age groups.

RESULTS
During the 6-year period 1660 cases of cancer of the oesophagus were reported to the Danish Cancer Registry. 580 of these 1660 were adenocarcinomas and 1080 squamous cell cancers of the oesophagus (Table 1).
It was possible to retrieve relevant medical data for 526 (90.7%) of these 580 cases of adenocarcinoma of the oesophagus from the Danish Cancer Registry, including the results of endoscopy with biopsy in 487. Subsequently 507 of the 580 (87.2%) adenocarcinomas could be classified, 349 as ACO and 158 as AGC. However, 73 cases (12.8%) were unclassifiable (Figure 1). The quantitative relationships between classified and unclassified cases by age for the two genders were illustrated in Figures 2 and 3.
Figure 1. An organization chart showing the differentiation of the 580 patients with adenocarcinoma of the oesophagus into adenocarcinomas limited to the oesophagus (ACO), the gastric cardia (AGC) and patients unclassified because of absent or insufficiently detailed medical data.
Figure 2. The incidence rates of: all adenocarcinomas of the oesophagus (All Adeno Ca), adenocarcinomas in BO and limited to the oesophagus (ACO), adenocarcinoma of the gastric cardia (AGC) and unclassified cases in males. The majority of unclassified cases occurred after the age of 75, all three categories showed the same steep decline in the 85+ age band. These unclassified cases (Unclassified) implied that the incidence rates of ACO and AGC in the over 75 age groups were, in fact, higher than reported.

Figure 3. The incidence rates of: all adenocarcinomas of the oesophagus (All Adeno Ca), adenocarcinomas in BO and limited to the esophagus (ACO), adenocarcinoma of the gastric cardia (AGC) and unclassified cases (Unclassified) in females, note that the scale of the y-axis is 1/5th of that in males.
Table 2 The Incidence Rates of Adenocarcinoma, Limited to the Oesophagus (ACO) by Age and Gender, Denmark 1987-92

<table>
<thead>
<tr>
<th>Age Bands</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population 1990 n.</td>
<td>ACO 87-92 n.</td>
</tr>
<tr>
<td>30-34</td>
<td>190 126</td>
<td>1</td>
</tr>
<tr>
<td>35-39</td>
<td>188 443</td>
<td>1</td>
</tr>
<tr>
<td>40-44</td>
<td>210 290</td>
<td>7</td>
</tr>
<tr>
<td>45-49</td>
<td>178 635</td>
<td>11</td>
</tr>
<tr>
<td>50-54</td>
<td>141 558</td>
<td>21</td>
</tr>
<tr>
<td>55-59</td>
<td>124 390</td>
<td>24</td>
</tr>
<tr>
<td>60-64</td>
<td>118 163</td>
<td>32</td>
</tr>
<tr>
<td>65-69</td>
<td>112 767</td>
<td>46</td>
</tr>
<tr>
<td>70-74</td>
<td>87 960</td>
<td>45</td>
</tr>
<tr>
<td>75-79</td>
<td>68 013</td>
<td>54</td>
</tr>
<tr>
<td>80-84</td>
<td>38 892</td>
<td>33</td>
</tr>
<tr>
<td>85+</td>
<td>23 026</td>
<td>10</td>
</tr>
<tr>
<td>Totals</td>
<td>285</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. The incidence rates of adenocarcinomas limited to the oesophagus (ACO) by age and gender. In males the steep rise in incidence rates levelled off from the age band 75-79 and showed a steep decline in the 85+ age band. In females the rise in incidence rates was much less pronounced, as was the fall in the 85+ age band. The gender ratio was 5.9:1, 95% C.I. 4.4-7.9.
The incidence rates for ACO were displayed in Table 2 and graphically represented in Figure 3. In both genders they increased with age, for males from 0.09/10^5 in the 30-34 age-band to 14.14/10^5 (95% CI 10.0 - 19.9) in the 80-84 age-band, then falling to 7.2/10^5 (95% C.I. 3.9-13.4/10^5) in the 85+ age-band and for females from 0.19/10^5 in the 45-49 age-band to 2.79/10^5 (95% CI 1.6-4.9) in the 80-84 age-band, then falling to 2.43/10^5 (95% CI 1.3-5.1) in the 85+ age-band. The log linear regression curves ran parallel, indicating a similar pattern for both genders. The resulting age-adjusted male: female OR was 5.9:1, 95% CI 4.4-7.9.

The incidence rates of ACO and AGC were graphically represented in Figure 5. Those of AGC also increased with age in both genders, for males from 0.32/10^5 in the 35-39 age band to 7.65/10^5 in the 75-79 age band and then falling to 1.74/10^5 in the 85+ age band and for females from 0.12/10^5 in the 40-44 age band to 2.01/10^5 and then falling to 1.09/10^5 in the 85+ age band. The age adjusted male: female OR was 4.26:1, 95% CI 2.94-6.17.

The incidence rates of SCC by age and gender were graphically represented in Figure 6. The incidence rates of SCC rose for males from 0.35/10^5 in the 35-39 age band to 25.3/10^5 (95% CI 19.6-32.6) in the 80-84 age band, then falling to 23.9/10^5 (95% CI 17-33.6) in the 85+ age band and for females from 0.17/10^5 in the 40-44 age band, rising uninterruptedly to 18.6/10^5 (95% CI 14.4-24.9) in the 85+ age-band. The log linear regression curves ran parallel, indicating a similar pattern for both genders. The age-adjusted male: female OR was 2.46, 95% CI 2.17-2.78.

The age and gender specific log-transformed parabolas for ACO and SCC incidence rates just achieved significant differences (p=0.034). However, comparison of these parabolas for the over 60-year age groups (comprising 277 cases of ACO and 824 of SCC) revealed far clearer differences (p<0.001), with a continuing rise for SCC but a levelling off, followed by a decline for ACO from around the age of 80 years. (Figure 7)
Figure 6. The incidence rates of squamous cell carcinomas of the oesophagus (SCC) by age and gender. In males there was a slight decline in the 85+ age band, which was compensated by a steep rise in females in the 85+ age band resulting in a continuing rise for all age groups combined. The gender ratio was 2.46, 95% C.I. 2.17-2.78.

Figure 7. The age and gender specific logarithmic transformation parabolas for ACO and SCC for the over-60 age group comprising 277 cases of ACO and 824 of SCC, showing the significant differences in the incidence patterns (p < 0.001), specifically in the over-80 age group.
DISCUSSION

This study set out to establish the age and gender specific incidence rates of adenocarcinoma limited to the oesophagus (ACO) over a 6-year period within a defined geographical area (Denmark) comprising about 5 million inhabitants. Its strength lay in that 87% of all cases of adenocarcinoma of the oesophagus were defined as either ACO or adenocarcinoma of the gastric cardia (AGC) on the basis of adequate clinical data by a panel of experts.2 The great majority of the unclassified cases were found in the over-75 age groups (Figures 1 and 2). In addition, it looked for differences between the patterns of the incidence rates of ACO and of squamous cell carcinoma of the oesophagus (SCC).

Within the group of 580 cases of adenocarcinoma of the oesophagus registered over the six year period, 349 cases were identified as ACO.

The age and gender specific incidence rates of ACO (Figure 3) showed an initial exponential rise, particularly marked in males, gradually levelling off around the age of 80 and finally declining steeply in the over-85 age band. The age-adjusted male: female incidence ratio was 5.9:1. This gender ratio was slightly lower than the age-adjusted 6 to 8:1 ratio found in a recent update of the National Cancer Institute’s Surveillance, Epidemiology, and End Results (SEER) data between 1977-96. 30 Both gender ratios were, however, unusually high for a non-genital tumour.

The declining ACO incidence rates in the oldest age groups were not in accordance with accepted multistage models indicating that the age-specific incidence of many human cancers increases roughly with a power of age.31-33

An earlier analysis of SEER data from 1972-82 by Yang et al.21 also reported age specific rises in ACO incidence rates without the declining incidence rates in the over-80 age group, which was however, shown graphically in a subsequent analysis of SEER data, undifferentiated for gender, for the years between 1987-96. 30 The pattern of the AGC incidence rates mirrored that of ACO to a certain degree (Figure 4) although in males the age specific rise in incidence rates was far lower than in ACO. The AGC male: female ratio of 4.26: 1 was, as expected, lower than in ACO.

The ACO: AGC incidence rate ratio was 2.2: 1, contrary to that found in the SEER study where AGC rates exceeded those of ACO, although here a secular trend towards parity was observed30, 34. The difference between the Danish and the US findings may have been due to a better differentiation by the expert panel or to geographical variations34.

The 1080 cases of SCC presented a markedly different incidence pattern from that of ACO, with the age specific rise in incidence continuing into the oldest age bands (Figure 5) and a male: female incidence ratio of 2.46:1, less than half of that found in ACO.

In the over 60-year age group this difference between the rise in the age specific incidence rates of SCC, continuing into the 85+ age band and the declining pattern from age-80 in ACO, was found to be highly significant (p< 0.001) (Figure 6). The latter analysis included 63 cases of ACO and 196 cases of SCC over the age of 80.

In theory this declining ACO incidence rate could have resulted from less exposure to carcinogens, i.e. tobacco and alcohol, in the 80+ cohort. The absence of a similar effect on the 80+ SCC incidence rate clearly contradicts this hypothesis.

We propose that these clear differences in the patterns of the 80+ incidence rates of ACO and SCC were the result of the limited size of the 80+ BO sub-population at risk, thus restricting the number of cases of ACO and thereby reversing the expected age specific exponential rise in the incidence rate of ACO in the general population.

How could the size of the 80+ BO sub-population have been limited? Within the 80+ BO sub-population itself, the age specific ACO incidence rate was likely to have continued to rise exponentially. Therefore, in theory, the ACO incidence rate within the BO sub-population
could have approached a level of 100% between the ages of 80 to 84, thus preventing further increases.

This hypothesis must be rejected as three studies of the prevalence of BO in clinical endoscopy found substantial numbers of patients with BO, but free of ACO, in their eighth and even their ninth decades. However, all three studies did find falling prevalence rates of BO in the over-80 year age-group \(^{11,35,36}\). The falling incidence of ACO in the elderly could also have been caused by decreasing diagnostic effort or competing causes of death in this age group. However, the absence of a similar decline in the over-80 incidence rate of SCC led us to reject under diagnosis or under-reporting as its cause. In view of the strong relationship between SCC and smoking and drinking habits, \(^{37,38}\) the incidence rates of symptomatic SCC, especially in males, were almost certainly curtailed by competing causes of death such as cardiovascular diseases and other cancers. As these lifestyle factors are of relatively smaller importance in the aetiology of ACO, \(^{37-41}\) such competing causes of death were likely to have had the greatest negative impact on SCC incidence rates.

Our ACO and AGC incidence rates can be criticized on the grounds that 12% of the cases remained unclassified. However, as shown in Figures 1 and 2, the great majority of these unclassified cases occurred in the over-75 age groups, possibly because only limited diagnostic efforts were applied as these patients were only considered suitable for palliation. The incidence rates of ACO and AGC in these older age groups were therefore higher than given in Figures 3 and 4 but, at least for males, the sharp decrease in the 85+ age band remained unchanged (Figure 1).

Our conclusions were based on the assumption that, in practice, all cases of adenocarcinoma of the oesophagus, not originating from the true gastric cardia, arise in BO\(^{42-46}\). It implied a strict ‘entry criterion’, BO, which could only be met by a small, but ill defined fraction of the population. This in contrast to SCC, which had no such ‘entry criterion’. We cite a number of additional arguments for this assumption. With the exception of the unexplained findings in a single population based study, \(^{47}\) ACO has exclusively been seen to arise prospectively in BO\(^{48,49}\). Identifying BO in ACO requires both experience and devotion to detail. In our group of 349 patients with ACO, derived from a Cancer Registry, BO was only diagnosed in 19%. \(^{2}\) In a study by Lagergren et al., where all patients were examined according to standardized protocols, BO was detected in 62%\(^{5}\). That a 100% result was not achieved was explained by Sabel et al. who found that the only difference between adenocarcinomas of the oesophagus with and without visible BO tissue was that the latter were larger and more advanced, suggesting that these aggressive tumours had overgrown all the original BO.\(^{50}\) This is especially true for tumours arising in short segment BO\(^{45,46}\). In addition, if adenocarcinomas could arise in any part of the oesophagus, cases should be found with a circumferential area of squamous epithelium between the tumour and the squamocolumnar junction. However, in classifying thousands of oesophageal cancers for the Rotterdam Oesophageal Cancer Group over a 25 year period, only one such case was seen. (J. Dees, 2003, personal communication). Although in theory cases of ACO could arise without an intervening stage of BO, these rare events would not affect our conclusions.

We suggest that the greater than twofold difference in gender ratios for two tumours arising in the same organ again reflected the effect of BO prevalence. This is known to have a male: female ratio of between 2 and 4: 1\(^{11,35,36,51}\). Obviously, the gender ratio in SCC may also have been influenced by gender related differences in lifestyles. In fact, in females the incidence of SCC continued to rise to 24.73/10^5 in the 90+ age group (n. SCC: 21). Our hypothesis explaining the declining incidence rates of ACO in the elderly obviously raised the question as to the cause of the age specific decline in the prevalence of BO.
We submit three hypothetical mechanisms, the excess mortality from ACO, obviously limited to the BO population, an increased susceptibility to other, non-related causes of death caused by an unhealthy life-style\textsuperscript{52} and a birth-cohort effect \textsuperscript{53}. This last mechanism would imply that over the twentieth century birth-cohorts, consisting of all Danes born in consecutive 5-year periods, would have demonstrated a secular increase in the prevalence rate of BO, with the lowest rates in the oldest birth-cohorts.

However, in the absence of further data, we were unable to choose between these three mechanism, although the finding of a birth cohort effect on the incidence rates of ACO in a recent review of the SEER results from the United States would tend to support the third mechanism\textsuperscript{30}.

The most important practical aspect of our study is that the currently often quoted estimate of the annual ACO incidence rate of 0.5% in patients with BO \textsuperscript{54} cannot be applied to individual BO patients as we have shown their cancer risk to be linked to both age and gender. There is urgent need for new risk estimates taking these factors into account. There are also consequences for the economic modelling of BO surveillance, which already has to revise age limits as oesophagectomy is no longer the sole available treatment for HGD and early ACO\textsuperscript{55, 56}. Consequently, it would be logical to introduce both age and gender into the calculations of the optimal frequency of surveillance endoscopies, especially with advanced age no longer being a ground for exclusion.

In conclusion, this Danish population based demographic study described the age and gender specific incidence rates of ACO. It demonstrated that their age specific exponential rise gradually levelled off around the age of 80 and actually fell in the over-85 age group. AGC demonstrated a similar pattern, this in contrast to the continuing rise in incidence rates of SCC in the elderly. Therefore, the falling incidence rate in ACO was not caused by under diagnosis in the elderly but most likely demonstrated the effect of an age specific decline in the prevalence rate of BO in the elderly. In ACO a 6:1 gender ratio was found, more than twice the 2.5:1 gender ratio found in SCC.

This difference again reflected the influence of BO prevalence rates on the incidence rates of ACO, in this case the 2:1 or greater gender ratio in the prevalence of BO.

References

Chapter 5

MODDELING A POPULATION WITH BARRETT’S OESOPHAGUS
FROM OESOPHAGEAL ADENOCARCINOMA
INCIDENCE DATA

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**ABSTRACT**

**Background:** A recent study of the adenocarcinoma of the oesophagus (ACO) incidence rates in Denmark revealed a steep fall in the over-80 population, interpreted as the result of a declining prevalence of Barrett’s oesophagus (BO) in this age group for which three hypotheses were advanced: the specific mortality from ACO and, superimposed, either excess mortality from causes of death unrelated to ACO or a birth cohort effect.

**Aims:** To create models estimating the BO population fitting each of these 3 hypotheses, to select the most plausible hypothesis and gain insight into the Danish BO population.

**Methods:** Models were designed for three hypotheses, conforming to the generally accepted 0.4-0.5% annual ACO incidence in BO patients. These models employed expectation-maximization algorithms, Danish life tables and the observed ACO incidence rates. The models enabled the estimation of a BO population for each hypothesis.

**Results:** After testing against set criteria, the most plausible model was found to be that describing a birth cohort effect which predicted a ± 5% annual rise in the prevalence of BO and consequently, in the incidence rate of ACO in Denmark. This prediction was borne out over the subsequent decade.

**Conclusions:** This rising ACO incidence rate is likely to continue into the foreseeable future. The use of EM algorithms enabled a first estimate of the BO population at risk of ACO although, owing to the limitations imposed by the models, the age and gender specific ACO risk for the entire Danish BO population could not as yet be ascertained.
INTRODUCTION

Barrett’s oesophagus (BO), or columnar metaplasia of the distal oesophageal mucosa, is a well recognized pre-malignant condition\textsuperscript{1,2} and presumably the precursor of practically all cases of adenocarcinoma limited to the oesophagus (ACO) \textsuperscript{2-5}. The incidence of ACO is rising rapidly in the Western World\textsuperscript{6-11}.

In a recent study, we described the age and gender specific ACO incidence rates for the population of Denmark around 1990 in which an unexpected steep fall of the ACO incidence in elderly over 80 years of age was observed. We ascribed this to a declining BO prevalence in this age group. Three hypotheses were advanced to explain this declining BO prevalence. These hypotheses were: 1) the increased mortality rate for the BO population, as compared to the general Danish population, caused by the mortality from ACO, uniquely inherent to the BO population and, in addition to this specific ACO mortality, 2) excess mortality from other causes of death due to a hypothetical inferior state of health of the BO population, or 3) a birth cohort effect, i.e. a secular increase in the prevalence rate of BO in consecutive birth cohorts during the twentieth century, resulting in relatively smaller BO populations in the elderly\textsuperscript{12}.

However, as there were no data on the size and composition of the Danish BO population, it was impossible to decide which of these 3 hypotheses was the most plausible. We hypothesized that the age-specific prevalence of BO in Denmark could be deduced from a combination of our observed incidence rates of ACO and the published age and gender specific incidence rates of ACO for BO derived from large BO populations with an adequate follow up period. We set out to estimate the size of its BO populations for each of the above mentioned three hypotheses, by means of models based on expectation-maximization (EM) algorithms. In each model the age and gender specific incidence rates of ACO for its estimated BO population had to approach the observed ACO incidence rates as closely as possible.

Finally, the outcomes of the three models were tested against set criteria to select the most plausible hypothesis.

METHODS

Unfortunately in the absence of adequate data on the age and gender specific prevalence of BO, we had to make do with the generally accepted annual incidence of ACO in patients with BO ranging around 0.5\textsuperscript{13, 14}. This relatively low ±0.5% annual incidence rate of ACO implied that, within any given group of BO patients, the incidence of ACO must be spread over a considerable period of time. This presumed time spread resulted from the combination of a wide variety in the ages of onset of BO\textsuperscript{15, 16} and, in analogy with chronic inflammatory diseases such as ulcerative colitis\textsuperscript{17}, an incubation period of up to several decades between the onset of BO and symptomatic ACO. The length of the incubation period introduced a second unknown variable into the models, a third being the age at onset of BO. These problems were overcome by defining our BO population as having acquired BO before the age of 46 and by setting the incubation period at over 30 years. Consequently, very few individuals acquiring BO after the age of 45 would survive to develop symptomatic ACO. Although not conforming to reality, these restrictions enabled us to construct the three models.

Models.

Input data for all models were the observed ACO incidence rates and Danish life tables. Models were designed for the 3 hypotheses, model 1, the specific mortality from ACO, model 2, the excess mortality from causes of death unrelated to ACO and model 3, the birth cohort effect. Each estimated the size of the age and gender specific of the BO population
for each age band over the age of 45, further referred to as the modelled BO population. All BO patients were defined as having acquired BO before the age of 46. The 45-year age limit was chosen because only very few cases of ACO occurred before this age. In all 3 models the age specific decline in the BO population was assumed to have been more rapid than that of the general Danish population because of the inherent extra mortality from ACO, unique to the BO population. In model 1, the basic model, this formed the only source of the more rapid decline. In addition, model 2 included excess mortality from causes of death unrelated to ACO and model 3 the birth cohort effect.

In all models the variables to be calculated were the size of the modelled BO population by age and gender for each age band and the resulting ACO incidence rates which had to approach the observed ACO incidence rates as closely as possible. The additional variables to be calculated were in model 1 the size of the modelled BO population as a percentage of the general population at the age of 45 (the modelled BO population between 45 and 75), in model 2 the size of the excess mortality and in model 3 the annual percentage growth of the BO population over the previous decades, the birth cohort phenomenon. For models 2 and 3, the unknown size of the modelled BO population between 45 and 75 first had to be estimated by introducing a variety of percentages within a range derived from the literature of about 0.5% per year until a result emerged within the target range (see under External criteria for judging the plausibility of the three models below). To compensate for the incomplete ascertainment of cases of ACO in our previous incidence study, amounting to at least 13%, the number of cases of ACO entered in each 5-year age band in the present study was the number actually observed during a six year period of the previous study.

**The EM algorithm**
The estimates of the age and gender specific prevalence rates of the modelled BO population for each model were calculated through a so called expectation-maximization (EM) algorithm which here attempted to fit an internally consistent description of the BO population as closely as possible to the observed population ACO incidence. Data entered into the EM algorithm were derived from the incidence rates of ACO for the population of Denmark between 1987-92 and the Danish life tables for the years 1989-90. An exact description of the EM algorithm and the resulting models can be found in the Appendix.

The EM algorithm started with a rough estimate of the fraction of the general population with BO, for instance 1%, for each age band. This percentage provided the number of people at risk for ACO, i.e. the modelled BO population, by age band which was then used to estimate an exponential curve describing the increasing ACO incidence rate by age. The actually observed numbers of ACO by age were used as outcomes for this log linear regression. This first estimate of the incidence of ACO by age in the modelled BO population, 1% in this example, enabled the estimation of the differences in survival between the modelled BO population and the general population. The comparison of these two survival curves then resulted in an improved estimate of the modelled BO population in each age band and this was used to estimate a new exponential curve describing the increasing ACO incidence rate by age. All these steps were repeated until they eventually led to a stable, optimal solution.

**Assumptions.**
In order to keep the models manageable five assumptions had to be made. These were:
1. For models 2, and 3; a 2:1 male: female gender ratio for the modelled BO prevalence at age-45.
2. That all cases of ACO originated in BO.
3. That the age specific incidence rates of ACO within the BO population would increase exponentially\textsuperscript{26-28}.
4. That the ‘incubation’ period between developing BO and symptomatic ACO was so long that in practice an insignificant number of individuals acquiring BO after age 45 survived to manifest symptomatic ACO.
5. That all cases of ACO were removed from the modelled BO population as the risk had materialised.

\textit{Set criteria for judging the plausibility of the three models}

The main criterion for all models was a target range of ACO incidence rates in BO patients based on the available literature\textsuperscript{13, 14, 29}. We assumed that most of the patients with BO, included in the surveys on which these estimates were based, were between the ages of 45-75. As the available estimates did not differentiate for age or gender, we were obliged to combine the results of the ACO incidence rates estimated by each model for both genders. We therefore chose the average of the combined male and female ACO incidence rates for the BO population between ages 45-75, as estimated by each model, to represent its outcome for comparison with the target range. This average figure, further referred to as the modelled ACO incidence rate\textsuperscript{45-75}, was calculated from the sum of the person-years observed for the male and female modelled BO population between the ages of 45 and 75 and the sum of the number of cases of ACO estimated by each model for these same age groups. The target range for the modelled ACO incidence rate\textsuperscript{45-75} was set at between 0.4/100\textsuperscript{14} and 0.5/100 person-years\textsuperscript{13}.

Additional criteria were, for the basic model 1, the plausibility of the prevalence rates of the modelled BO population between 45 and 75\textsuperscript{18, 19} resulting from this model and for model 2 the height of the excess mortality.

For model 3, an additional criterion was the annual rate of increase in the ACO incidence compared with the available data on rise in incidence of adenocarcinoma of the oesophagus, undifferentiated for ACO and adenocarcinoma of the gastric cardia (AGC), observed over the 13-year period, 1987-99.

\textbf{RESULTS.}

The results of the three EM algorithms are shown in Table 1. All three models generated estimated ACO incidence rates (column 9 in tables 2a and 2b) which fitted well with the observed ACO incidence rates (column 3), (Scaled deviance of 3.407 to 3.955 for 10-3 = 7 degrees of freedom). This meant that choosing the most plausible model had to be based on the results of the modelled ACO incidence rate\textsuperscript{45-75} and the set criteria listed previously.

In the basic model 1, the extra attrition of the modelled BO population, as compared to the general population, was caused by mortality from ACO. The modelled ACO incidence rate\textsuperscript{45-75} was 1.36/100 person-years. This fell well outside the target range of between 0.4 and 0.5/100 person years and in addition, the modelled BO populations between 45 and 75 of 0.387\% for males and 0.079\% for females were considered highly implausible\textsuperscript{18, 19}. Model 1 was therefore rejected.

Model 2, with an optimal modelled BO population between 45 and 75 of 1\% in males and 0.5\% in females and which, in addition to ACO, included excess mortality unrelated to ACO, produced a modelled ACO incidence rate\textsuperscript{45-75} of 0.41/100 person-years, within the target range. However, the calculated excess mortality for males of 51\% (95\% C.I. 26-76\%) and for females of 76\% (95\% C.I. 3-155\%) was, after weighing up the available literature\textsuperscript{30-34}, considered too high. Although conforming to the set target modelled ACO incidence rate\textsuperscript{45-75}, we therefore considered model 2 to be only moderately plausible.
### Table 1. The Results of the Three EM Algorithms

<table>
<thead>
<tr>
<th>Model</th>
<th>Modelled ACO incidence rate&lt;sup&gt;45-75&lt;/sup&gt;</th>
<th>The modelled BO population at 45</th>
<th>Extra mortality from unrelated causes</th>
<th>Birth cohort effect, annual increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.36</td>
<td>0.387%</td>
<td>0.079%</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.41</td>
<td>1%</td>
<td>51%&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>76%&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.42</td>
<td>1.5%</td>
<td>4.4%</td>
<td>6.3%</td>
</tr>
</tbody>
</table>

Abbreviations: EM = expectation maximisation; NA = not applicable; ACO = adenocarcinoma of the oesophagus; BO = Barrett’s oesophagus.

<sup>1)</sup> 95% CI 26-76%; <sup>2)</sup> 95% CI 3-155%.

### Table 2a. The Modelled BO Population and its Estimated ACO Incidences calculated for a BO Prevalence of 1.5% of the General Population at Age 45

<table>
<thead>
<tr>
<th>Age Bands</th>
<th>Male population number&lt;sup&gt;1990&lt;/sup&gt;</th>
<th>Observed ACO incidence n/5 yrs.</th>
<th>Non-BO population percentage survival</th>
<th>Modelled BO pop percentage survival</th>
<th>Modelled BO population percentage</th>
<th>Modelled BO population numbers</th>
<th>Modelled ACO incidence/100 pers.yrs.</th>
<th>Estimated ACO incidence n/5 yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Age 45</td>
<td>178 635</td>
<td>100%</td>
<td>100%</td>
<td>1.5%</td>
<td>2 679.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-49</td>
<td>178 635</td>
<td>11</td>
<td>98.7</td>
<td>98.4</td>
<td>1.377</td>
<td>2 459</td>
<td>0.11</td>
<td>13.4</td>
</tr>
<tr>
<td>50-54</td>
<td>141 558</td>
<td>21</td>
<td>95.7</td>
<td>94.7</td>
<td>1.104</td>
<td>1 563</td>
<td>0.21</td>
<td>16.6</td>
</tr>
<tr>
<td>55-59</td>
<td>124 390</td>
<td>24</td>
<td>90.7</td>
<td>88.4</td>
<td>0.879</td>
<td>1 094</td>
<td>0.42</td>
<td>22.7</td>
</tr>
<tr>
<td>60-64</td>
<td>118 163</td>
<td>32</td>
<td>83.4</td>
<td>78.8</td>
<td>0.689</td>
<td>815</td>
<td>0.81</td>
<td>33.1</td>
</tr>
<tr>
<td>65-69</td>
<td>112 767</td>
<td>46</td>
<td>72.9</td>
<td>65.0</td>
<td>0.525</td>
<td>593</td>
<td>1.59</td>
<td>47.0</td>
</tr>
<tr>
<td>70-74</td>
<td>87 960</td>
<td>45</td>
<td>59.0</td>
<td>47.1</td>
<td>0.379</td>
<td>333</td>
<td>3.1</td>
<td>51.6</td>
</tr>
<tr>
<td>75-79</td>
<td>68 013</td>
<td>54</td>
<td>42.5</td>
<td>27.4</td>
<td>0.246</td>
<td>167</td>
<td>6.05</td>
<td>50.6</td>
</tr>
<tr>
<td>80-84</td>
<td>38 892</td>
<td>33</td>
<td>25.6</td>
<td>11.0</td>
<td>0.130</td>
<td>51</td>
<td>11.8</td>
<td>29.9</td>
</tr>
<tr>
<td>85+</td>
<td>22 026</td>
<td>9</td>
<td>15.7</td>
<td>2.6</td>
<td>0.038</td>
<td>8</td>
<td>24.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Totals</td>
<td>982 404</td>
<td>275</td>
<td></td>
<td></td>
<td></td>
<td>7 083</td>
<td></td>
<td>275.03</td>
</tr>
</tbody>
</table>
Table 2b. The Modelled BO Population and its Estimated ACO Incidences calculated for a BO Prevalence of 0.75% of the General Population at Age 45

| Model 3, Females |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age Bands       | Female Population 1990 numbers | Observed ACO incidence n/5 yrs. | Non-BO population survival | Modelled BO pop. percentage survival | Modelled BO population percentage | Modelled BO population numbers | Modelled ACO incidence/100 pers.yrs. | Estimated ACO incidence n/5 yrs. |
| Age             | 173 493          | 2               | 100%            | 100%            | 0.75%           | 1300            | 0.03            | 1.6              |
| 45-49           | 173 493          | 2               | 99.1            | 99.1            | 0.666           | 1156            | 0.06            | 2.2              |
| 50-54           | 141 613          | 1               | 97.1            | 96.7            | 0.491           | 695             | 0.13            | 3.1              |
| 55-59           | 129 484          | 4               | 93.7            | 93.0            | 0.360           | 467             | 0.29            | 4.9              |
| 60-64           | 128 697          | 3               | 89.0            | 87.4            | 0.263           | 339             | 0.63            | 7.9              |
| 65-69           | 132 065          | 8               | 82.4            | 79.2            | 0.190           | 251             | 0.89            | 12.1             |
| 70-74           | 111 463          | 15              | 73.4            | 67.1            | 0.133           | 149             | 1.37            | 10.2             |
| 75-79           | 99 711           | 11              | 61.1            | 50.5            | 0.089           | 88              | 3.0             | 13.1             |
| 80-84           | 71 656           | 12              | 44.9            | 29.8            | 0.052           | 38              | 6.45            | 12.1             |
| 85+             | 51 914           | 7               | 36.8            | 12.8            | 0.019           | 10              | 15.4            | 7.8              |
| Totals          | 1 040 096        | 63              |                 |                 |                 |                 |                 | 63               |

Abbreviations; ACO = adenocarcinoma of the oesophagus; Barrett’s oesophagus = BO

Tables 2a and 2b. explanation of headings.

The modelled BO population: The BO population estimated by the model for each age band. All data are presented by 5 year age bands. The numbers refer to columns.


3. Observed ACO incidence n/5 yrs: The observed number of cases of ACO between 1987-92.

4. Non-BO population percentage survival: The percentage of the general population surviving according to Danish life tables for 1990, data used to estimate the survival of the population without BO.

5. The modelled BO population percentage survival: The estimated percentage of the BO population surviving as estimated by the model in comparison with the general population (column 4).

6. The modelled BO population percentage: At age 45, the optimal percentage of the general population with BO employed by the model. Subsequently, the percentage of this BO population that was still alive at age 45 according to column 5 for each age band.

7. The modelled BO population numbers: The estimated size of the BO population, column 6 x column 2 divided by 100.

8. Individual ACO incidence: The incidence rate of ACO estimated by the model for the modelled BO population per 100 person-years.

9. Estimated ACO incidence/5 yrs: The number of cases of ACO between 1987-92 estimated by the model, (col7 x 5) /col.8 , calculated to approach the observed number as closely as possible. Compare with column 3.

Model 3, the birth cohort effect, with an optimal modelled BO population between 45 and 75 of 1.5% in males and 0.75% in females, produced a modelled ACO incidence rate of 0.42/100 person-years, within the target range. The birth cohort effect, i.e. the annual rise
in the prevalence of BO in the population at large, was estimated at 4.4% for males, 6.3% for females and 5.1% for both genders combined. Assuming that this annual rise in BO prevalence was paralleled by a similar annual rise in the incidence of ACO, this should have resulted in the ACO incidence increasing by 4.6-5.1% per annum for both genders combined in the over-45 population of Denmark. This prediction was tested for our study period and the last 3 years for which incidence data were available.

The incidence of all adenocarcinomas of the oesophagus, not differentiated for ACO and AGC, over the 6-year period 1987-1992, was 580 (465 males, 115 females) and over the 3-year period 1997-1999, 428 (354 males, 74 females). On the basis of Poisson regression, the observed annual increase in the incidence of ACO and AGC combined for the over-45 population of both genders between 1987 and 1999 actually amounted to 4.74%, 95% C.I. 3.28-6.22. Model 3 was therefore judged to be the most plausible. The detailed results of model 3 are shown in Tables 2a and 2b and illustrated in Figures 1 and 2.

**Discussion**

The incidence of both Barrett’s esophagus (BO) and adenocarcinoma of the oesophagus (ACO) is rapidly increasing in Western populations. Cost-utility studies have suggested confining endoscopic surveillance to patients with BO experiencing an expected risk of ACO of at least 0.5/100 person-years. However, this advice is gratuitous as there are currently no clinical criteria for estimating the ACO risk for individual patients other than the presence of dysplastic histological changes, which already herald the latter stages of the BO cascade to ACO.

In contrast to other malignancies affecting the whole population (or just one gender) this risk cannot be estimated from the available population based incidence rates of ACO. This is because the essential denominator, the size and composition of the BO population, is currently unknown as there is currently only one BO prevalence studies in a large randomly selected population. However, the 16 cases of BO identified were too few to provide meaningful age and gender specific data. Our study represents a first step in an attempt to estimate this size and composition of the BO population from ACO incidence rates as these two factors are obviously interrelated. For our ACO data we chose our earlier study on the incidence rates of ACO in Denmark, which described an unexpected fall in the ACO incidence rates.

![Figure 1](image_url)

**Figure 1.**  
The incidence curves calculated on the basis of model 3 for BO prevalence rates of 1.5% for males and 0.75% for females resulting in a modelled ACO incidence rate of 0.42%.
incidence rate of the over-80 population around 1990. This was attributed to a declining prevalence rate of BO in this age group and for which 3 hypotheses were advanced. Three models, based on EM algorithms for these 3 hypotheses, were tested against external criteria, the most important of which was a modelled ACO incidence rate of between 0.4 and 0.5/100 person years. Model 1 was rejected as it did not conform to this and the other set criteria. Model 2, although conforming to the modelled ACO incidence rate was considered less plausible because of the height of the excess mortality involved. Whether such excess mortality exists is a matter of dispute. A fundamental problem is the symptom overlap with ischaemic heart disease, as was demonstrated in a recent UK population based study in which the calculated excess mortality lost its significance after correction for the presence of ischaemic heart disease. The BO cohort follow up study from Rotterdam, reporting an excess mortality of 50% included a large proportion of patients already suffering from severe unrelated diseases. Conflicting results were reported for the survival in a cohort of 117 BO patients from Olmsted County and an earlier study from the same centre. Two recent studies, from Germany and from Northern Ireland, failed to find an increased overall mortality in substantial cohorts of BO patients.

By conforming to all criteria, model 3, describing the birth cohort effect, was finally judged the most plausible. This conclusion was in accordance with the finding of a significant birth cohort effect in the incidence rates of ACO reported in a recent analysis of SEER data. The implications of the birth cohort effect may be far reaching. If the calculated age specific expansion of the Danish BO birth cohorts of around 4%-5% per annum continues, it will cause a similar rise in the incidence of ACO. This process is then likely to continue until the BO population achieves its maximum. The existence of a secular rise in the prevalence of BO was recently confirmed in a study from The Netherlands. Currently, the most important underlying causes for this mounting BO prevalence are thought to be the combination of the secular fall in the prevalence of Helicobacter pylori infection and the increasing prevalence of obesity in industrialized countries.
An intriguing alternative hypothesis was recently launched, focusing on the increasing nitrate consumption from nitrogenous fertilizers in the 20th century, specifically after World War II, resulting in high nitrite concentrations in the saliva. This nitrite, when encountering gastric acid, causes high concentrations of NO at the gastro-oesophageal junction which can act as a carcinogen. As the observed increased cancer rate manifests itself as adenocarcinoma and not as squamous cell carcinoma, this hypothesis requires an initial BO induction by NO, which is quite conceivable. Although this pathogenesis bears some of the hallmarks of a period effect, the birth cohort effect may have resulted from the fact that, in comparison with the younger birth cohort, the older birth cohorts experienced a smaller cumulative exposition.

Our method required several assumptions. To overcome the problem of the unknown date of onset of BO, we defined the modelled BO population as having developed BO before the age of 46. It should therefore be emphasized that the ACO incidence rates found in model 3 were only valid for a population which had acquired BO before the age of 46. As a result, with the exception of patients diagnosed with BO before this age, our incidence rate data as illustrated in figures 1 and 2 can only be employed as a worst case estimate for BO patients diagnosed at a later age. Two recent studies have shown a constant age specific rise in the BO prevalence, confirming that the majority of patients do indeed acquire BO at an advanced age.

Therefore, the majority of the BO population would have acquired BO after the age of 46 and consequently, the entire Danish BO population was obviously far larger than our modelled BO population. As this majority was not included in our model 3, this model could not predict their prognosis.

We also assumed a long incubation time between the onset of BO and symptomatic ACO based on the following considerations. First, although in theory the generally accepted 0.5% annual incidence of ACO in BO patients could result from random occurrences, the cascade: intestinal metaplasia without dysplasia to low-grade and finally to high-grade dysplasia and ACO implies a time frame which, even in the case of high-grade dysplasia, can still amount to over 7 years. Second, an analysis of data from the last of 3 consecutive reports from the Rotterdam study of 155 BO patients followed up for between 17 and 27 years and in which a constant ACO incidence rate of around 0.5 per 100 person years was observed, revealed that 44 survivors, diagnosed at an average age of 49.6 years, had experienced an average ACO free interval of 20 years (range 16.3-26.5 years). An incubation period of at least 20 years for individuals acquiring BO before age 46 is therefore plausible.

A basic assumption in our study was that practically all ACO arise in BO. This concept was recently challenged by Chang, citing studies by Bytzer and Lagergren who found BO in only 19% and 62% respectively of patients with ACO. However, the first study consisted of data from a cancer registry, while in the second all patients were examined according to standardized protocols, resulting in a far higher BO score. Sabel et al. found that the only difference between ACO with and without visible BO tissue was that the latter tumours were larger and more advanced, suggesting that these aggressive tumours had overgrown the whole original metaplastic surface. The final test for the existence of ACO without BO would be the finding of oesophageal adenocarcinomas originating from non-metaplastic columnar epithelium, i.e. sub-mucosal oesophageal mucous glands. A number of such cases were published in case series from the nineteen-sixties, each of which included only one case of ACO, suggesting that, at that time, this was the most common type of adenocarcinoma of the oesophagus. However, only two such cases have been published in the recent literature, indicating the impact of these tumours on current ACO epidemiology to be negligible. We therefore maintain BO to be an essential condition for the development of ACO and a valid basis for our estimates.
In conclusion, this study described a first attempt at estimating the hitherto invisible BO population of Denmark from ACO incidence data. It succeeded in estimating the size of a limited segment of the BO population by age and gender, by means of EM algorithms and established a cohort effect as the most plausible cause for an observed decrease in the elderly BO population. However, owing to the limitations imposed by the models employed, it was unable to ascertain the age and gender specific ACO incidence for the entire Danish BO population. This goal will require the development of entirely new models which admit the inclusion of a BO population whose age at onset of BO is based on realistic data. Our study also underlines the importance of developing techniques able to estimate the duration of the presence of BO in individual patients.
APPENDIX.

The mathematical models.
An expectation maximation model (EM) algorithm was constructed for the ACO incidence rates. EM algorithms are an iterative extension of regression models designed to handle a latent variable: a variable that cannot be observed explicitly; here the fraction of the population with BO. The Model-step (M-step) estimated the incidence curve, given a fraction at risk. This was used to calculate life tables for both the exposed (BO) and non-exposed (general) population. The Estimate-step (E-step) then combined these life tables to estimate the fraction at-risk which could be used again in the next M-step. Steps were iterated until an optimum was reached.

Model 1, the basic model
The first step (the M-step) estimated the exponential curve as

\[
\hat{Y}_a = \Phi_a P_a e^{\alpha + \beta X_a} ; Y \approx \text{Poisson}
\]

with:
\( \hat{Y}_a \) estimated incidence at age \( a \)
\( \Phi_a \) fraction with BO at age \( a \)
\( P_a \) total number of person-years at age \( a \)
\( e^{\alpha + \beta X_a} \) incidence rate within the group at risk

(\( \Phi_a \) was fixed in this step and \( \alpha \) and \( \beta \) were estimated)

In the second step the life tables for the BO and non-BO population were calculated:

\[
L_a = \prod_{\lambda=45,a} (1 - I_a)(1 - M_a)
\]

with:
\( L_a \) fraction surviving at age \( a \)
\( I_a \) incidence rate of ACO at age \( a \)
\( M_a \) background mortality at age \( a \)

The mortality from ACO was assumed to be both equal and simultaneous with the incidence and was obviously zero in the non-BO population. The background mortality was derived from the Danish life tables and not corrected for ACO mortality because of the very small contribution of ACO to total mortality.

Subsequently the fraction with BO at age 45 could be calculated from the combination of the life tables and the observed ACO incidence (the E-step):

\[
\hat{Y}_a = \frac{\Phi_{45} L'_a}{\Phi_{45} L'_a + (1 - \Phi_{45}) L_a} P_a I_a ; Y \approx \text{Poisson}
\]

with:
\( \hat{Y}_a \) estimated incidence rate at age \( a \)
\( \Phi_{45} \) fraction with BO at age 45
\( L'_a \) survival within the BO population
\( L_a \) survival within the non-BO population
\( P_a \) total number of person-years at age \( a \)
\( I_a \) incidence rate of ACO in the BO population at age \( a \)
(in this step the incidence rate of ACO was fixed and the single parameter $\Phi_{45}$ was estimated)
After estimating the fraction of the population with BO at age 45 the fraction at other ages could be calculated on the basis of the survival curves and the first step, estimating the exponential incidence curve within the BO population, could be repeated.

**Model 2, the effect of excess mortality from unrelated causes**

In the basic model ACO was the only extra cause of death in the BO population as compared to the general population. If, in addition, the BO population should suffer a higher death rate from unrelated causes this would add to the speed of depletion of the group at risk of ACO. The basic model was adapted to include the effect of such extra mortality on the BO population:

$$L_a = \prod_{\lambda=45}^{a} (1-I_a) \cdot (1-R.M_a)$$

with:

$R$ the relative risk of mortality in the BO population from unrelated causes of death.

To maintain a balance between the amount of available data and the number of parameters to be estimated, the prevalence rate of BO at age 45, $\Phi_{45}$ was set at 1% for males and 0.5% for females.

**Models 3, the cohort effect**

Another adaptation of the basic model 1 investigated the effect of an increase in the prevalence rate of BO over the decades before 1990. This would imply that the falling incidence rate of ACO in the elderly was not so much the result of depletion, but of a lower initial prevalence rate of BO in these age cohorts. This was achieved by extending another part of the basic model:

$$\hat{Y}_a = \frac{\Phi_{45} \cdot \rho^{-[a-45]} \cdot L'_a}{\Phi_{45} \cdot \rho^{-[a-45]} \cdot L'_a + (1-\Phi_{45}) \cdot L_a} \cdot P_a I_a ; \ Y \approx \text{Poisson}$$

with,

$\rho$ the relative annual increase of the fraction BO at age 45 before 1990.

Here the prevalence rates of BO at age 45 were set at 1.5% for males and 0.75% for females and $\rho$, $\alpha$ and $\beta$ were estimated.

Confidence intervals were calculated by the method for likelihood based confidence intervals$^{68}$.
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Chapter 6

DIFFERENTIAL TIME TRENDS IN THE INCIDENCE OF OESOPHAGUS-CARDIA ADENOCARCINOMA IN THE NETHERLANDS 1989-2003

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Submitted for publication
**Background:** In common with other industrialized countries, The Netherlands has experienced a rising incidence of oesophagus-cardia adenocarcinoma, raising the question of the respective roles of the two constituent tumours, i.e., adenocarcinoma of the oesophagus (ACO) and adenocarcinoma of the gastric cardia (AGC) in this rise.

**Aims:** To compare and contrast time trends for the incidence of ACO and AGC.

**Methods:** The trends in the 1989 to 2003 annual incidence rates by 3-year age classes and gender for ACO and AGC, as provided by the Netherlands Cancer Registry, were compared. In addition, these data were analyzed for age-period-cohort patterns.

**Results:** Over the 15-year period, the annual rise in the incidence rate of oesophagus-cardia adenocarcinoma was 2.6% for males and 1.2% for females. This was the net outcome of annual increases in ACO incidence (7.2% for males and 3.5% for females) and annual declines in AGC incidence of over 1% for both genders. For ACO and AGC, both genders exhibited a non-linear cohort pattern, whereas a non-linear period pattern was only observed for males with AGC.

**Conclusions:** The divergent time trends and age, period and cohort patterns between ACO and AGC clearly establish their epidemiological heterogeneity. The declining AGC incidence probably resulted from a secular decline in the number of male smokers and the two main etiological factors considered to be driving the rising ACO incidence not affecting the incidence of AGC.
INTRODUCTION

Before 1950, carcinoma of the oesophagus was practically always thought to be synonymous with epidermoid (squamous cell) carcinoma. The only rarely occurring adenocarcinomas were considered to have arisen from an upward extension of gastric cancers, oesophageal glands, or ectopic gastric mucosa in the proximal oesophagus (the “inlet patch”). However, from the early 1970’s adenocarcinomas of the oesophagus rapidly became more common. The recognition that such adenocarcinomas can, and mainly do develop in a columnar lined oesophagus (Barrett’s oesophagus) has created a great deal of interest in the factors causing Barrett’s oesophagus and its subsequent progression to adenocarcinoma. In addition, it enabled the current division of oesophagus-cardia adenocarcinoma into adenocarcinoma of the oesophagus, arising in Barrett’s oesophagus (ACO) and adenocarcinoma of the gastric cardia (AGC).

Over the past decades a steady rise in the incidence rates of oesophagus-cardia adenocarcinoma has been reported by cancer registries from the United States and Europe. However, whether this rise involved both ACO and AGC to the same extent has remained unclear, as most of these cancer registries were unable to distinguish between the two cancers. This is not surprising because, even for pathologists examining resected specimens, this fine distinction remains problematic. In Denmark, where Eurocim data had registered a 1.8:1 incidence ratio between AGC and ACO in males, a panel of experts, after reviewing the original clinical data over the same period, reversed this ratio to 1:2.4. The Netherlands is one of the countries with the highest incidence rates of both ACO and AGC. This finding obviously invited further investigation into the epidemiology of the constituent tumours of the oesophagus-cardia adenocarcinoma. Specifically, this study examined the epidemiological homogeneity of ACO and AGC in The Netherlands and their respective roles in the rising incidence of oesophagus-cardia adenocarcinoma by analyzing the differential time trends in the incidence of these two cancers and the presence of non-linear cohort-period patterns.

MATERIALS AND METHODS

The Netherlands, a country of 16 million inhabitants, is well served by a system of 9 regional Comprehensive Cancer Registries to which both hospital medical record departments and histology departments report all malignancies. This double case ascertainment ensures a high degree of accuracy. The Netherlands Cancer Registry in turn collates the data from all 9 Comprehensive Cancer Registries. The ascertainment of symptomatic oesophageal cancer in The Netherlands is likely to be high, as it practically always results in endoscopic and histological diagnosis for curative or palliative interventions. This Registry provided the annual age and gender specific incidence rates of ACO (ICD.10 C.15,3,4 and 5) and AGC (ICD10. C.116.0) for the 15-year period from 1989 to 2003, subdivided in 3-year age classes.

The time trends for each cancer were analyzed by log-linear Poisson regression models. For the estimation of the cohort models a mean year of birth was calculated for each 3-year age class. The estimated drift parameters constituted the annual percentage change in the incidence rates, corrected for age and population size. We used splines to test for non-linear period and cohort patterns. An observed annual percentage change may represent a period or a cohort effect but a choice between the two is only possible when one or the other is nonlinear. Therefore, instead of one exponential curve from the beginning to the end of the period, we extended the model to three exponential lines connected by knots at 1993 and 1998 for period estimates and 1926 and 1944 for those of birth cohort.
In both cases, the knots were placed at (approximately) one and two thirds of the time axis, without attempting to optimize their choice. Likelihood ratio tests (comparison of scaled deviances) showed whether significant non-linear period or cohort effects were present\textsuperscript{24, 25}. Male-female ratios were estimated from a model including incidence year, age class and gender.

RESULTS

Time trends by log-linear regression models for oesophagus-cardia adenocarcinoma, ACO and AGC

The incidence rates of oesophagus-cardia adenocarcinoma (ACO and AGC) rose from $7.4 \times 10^5$ to $10.0 \times 10^5$ (34%) in males, from $1.7 \times 10^5$ to $2.1 \times 10^5$ (25%) in females and from $9.1 \times 10^5$ to $12.1 \times 10^5$ (33%) in both genders combined over the period 1989-2003. The time trends for ACO and AGC for these years by gender are shown in Figure 1. The overall incidence rates of the two tumours over the total 15-year period were practically identical, for ACO $4.3 \times 10^5$ males and $0.96 \times 10^5$ females and for AGC $4.3 \times 10^5$ males and $0.92 \times 10^5$ females. However, while initially the male AGC incidence rate was far in excess of that of ACO, over the 15-year period the combination of a strongly rising ACO incidence rate with a downward trend in that of AGC, finally reversed this relationship starting from 1998 on. A similar, but less pronounced pattern was seen in females. The annual changes in the incidence rates, after correction for age, for both genders over the 15-year period 1989-2003 are shown in Table 1. The annual AGC incidence rates declined by -1.2% in females ($p=0.05$) and -1.7% in males ($p=0.0002$). This in contrast to the annual ACO incidence rates, which increased by 7.2% ($p<0.001$) for males but only 3.5% ($p=0.006$) for females. It can be concluded that ACO and AGC had contrary effects on the incidence of oesophagus-cardia adenocarcinomas.

The explanation for the 2-fold difference in the annual percentage increases in the ACO incidence rates between men and women is shown in Table 2. The annual percentage increases for both genders were highest in the 40-60 year age band, subsequently decreasing significantly for both genders in the 61-84 age bands ($p=0.03$). However, in males this decrease only amounted to 25% against 62% in females. Consequently the 1.2:1 male:female ratio in the 40-60 age band rose to 2.3:1 in the 61-84 age bands and this increased ratio was eventually to a large extent responsible for the differences between the genders in the annual rate of increase of 7.2% and 3.5% respectively.
Table 1. Annual Percentage Changes in the Incidence Rates of Adenocarcinoma of the Oesophagus and the Cardia by Gender 1989–2003

<table>
<thead>
<tr>
<th>Oesophageal Tumour</th>
<th>Gender</th>
<th>Numbers Observed</th>
<th>Incidence Rates/10^5</th>
<th>Annual % Change</th>
<th>95% CI</th>
<th>Annual Change</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>Males</td>
<td>4,949</td>
<td>4.43</td>
<td>+7.2%</td>
<td>+6.5 ;</td>
<td>+7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>1,589</td>
<td>0.96</td>
<td>+3.5%</td>
<td>+2.3 ;</td>
<td>+4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AGC</td>
<td>Males</td>
<td>4,863</td>
<td>4.34</td>
<td>–1.7%</td>
<td>–2.4 ;</td>
<td>–1.1</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>1,441</td>
<td>0.92</td>
<td>–1.2%</td>
<td>–2.4 ;</td>
<td>–0.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Source basic data: The Netherlands Cancer Registry

Table 1: the annual changes each represent the average value over the 15-year period. The incidence rates are corrected for age.

Table 2. The Annual Percentage Changes in the Incidence Rates of Adenocarcinoma of the Oesophagus by Age and Gender.

<table>
<thead>
<tr>
<th>Age bands</th>
<th>40-60</th>
<th>61-66</th>
<th>67-72</th>
<th>73-78</th>
<th>79-84</th>
<th>85+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>9.0%</td>
<td>5.9%</td>
<td>6.0%</td>
<td>7.0%</td>
<td>7.8%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Females</td>
<td>7.5%</td>
<td>4.8%</td>
<td>3.6%</td>
<td>0.6%</td>
<td>2.5%</td>
<td>4.3%</td>
</tr>
</tbody>
</table>

Table 2: the annual percentage increases corrected for age. The gender ratio for the age band 40-60 was 9% to 7.5%. Note the major differences between males and females after age 61. The male “dip” was short-lived, in females recovery only started around age 80. Over the 61-84 age bands the average male percentage increase was 6.7% against 2.9% for females, resulting in the overall 7.2% to 3.5% gender ratio.
Table 3. Cohort or Period Effects for Adenocarcinoma of the Oesophagus (ACO) and the Gastric Cardia (AGC) by Gender 1989-2003

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Gender</th>
<th>Cohort Effect</th>
<th>p value</th>
<th>Period Effect</th>
<th>p value</th>
<th>Annual % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>Males</td>
<td>+7.2</td>
<td>+7.1</td>
<td>+9.1</td>
<td>0.08</td>
<td>+6.8</td>
</tr>
<tr>
<td>ACO</td>
<td>Females</td>
<td>+2.1</td>
<td>+7.1</td>
<td>+4.0</td>
<td>0.006</td>
<td>+2.9</td>
</tr>
<tr>
<td>AGC</td>
<td>Males</td>
<td>+0.1</td>
<td>-3.0</td>
<td>-3.1</td>
<td>&lt;0.0002</td>
<td>+1.4</td>
</tr>
<tr>
<td>AGC</td>
<td>Females</td>
<td>-2.6</td>
<td>+0.1</td>
<td>+4.1</td>
<td>0.01</td>
<td>+1.4</td>
</tr>
</tbody>
</table>

Table 3: the age-cohort effects and age-period effects for ACO and AGC by gender. Cohort effects are tabulated separately for patients born before 1926, between 1926 to 1944 and after 1944. Period effects are tabulated separately for the years 1989 to 1993, 1994 to 1998 and 1999 to 2003. Statistical significance was calculated by comparing the values of these intervals.

**Age-period-cohort models**

The results of the cohort and period estimates are shown in Table 3. For each tumour, the mean changes in the annual incidence rates for each of three periods (before 1926, from 1926 to 1944 and after 1944) were differentiated by year of birth for cohort effects and by incidence year for period effects. A significant difference between the values for the three periods indicated the presence of a non-linear cohort or period effect. For example, the ACO incidence rates for males born before 1926, showed an annual change of 7.2% against 7.1% for patients born between 1926 and 1944, and increasing to 9.1% for patients born after 1944. However, the differences between these three percentages were not significant (p=0.08) (Figure 2). For the ACO period effect in males, the annual changes were +6.8 between 1989 and 1993, +6.5 between 1994 and 1998 and +8.4 between 1999 and 2003 (p =0.50) (Figure 3), i.e. there were no significant non-linear cohort or period effects in males with ACO. This in contrast to females with ACO where a significant non-linear cohort effect was seen (p=0.006), with the greatest increase in the 1926-44 cohorts (Figure 4), but here again there was no significant period effect (p=0.64).

For AGC, a non-linear cohort effect was found in males, p<0.0002, demonstrating a steady declining trend in cohorts born after 1926. This in contrast to the non-linear cohort effect in females, p=0.01, which demonstrated a rising trend in cohorts born after World War II (Figure5). Finally, for AGC a non-linear period effect in males, p=0.0001 (Figure 3), partly mirrored in females, p=0.3 suggested a decline caused by a period effect setting in around 1995.
Figure 2: the age-cohort model diagram for ACE in males. The seeming decline in the youngest birth cohorts is an artefact caused by a wide scatter in incidence rates. In spite of the large annual increases, especially in the birth cohorts born after 1944, the differences between the 3 sets of birth cohorts were not significant, \( p = 0.08 \).

Figure 3: the age-period model in males for both ACO, without a significant age-period effect, \( p = 0.5 \) and AGC with a very significant age-period effect. AGC incidences are seen to declining from around 1994 and this trend increases after 1999, \( p = 0.0001 \).
Figure 4: the age-cohort model diagram for ACO in females. Here there is a significant age-cohort effect, p=0.006.

Figure 5: the age-cohort model diagram for AGC in males and females. In males there is a very significant birth-cohort effect, demonstrating a steadily declining trend in AGC incidences in consecutive birth cohorts born after 1926, p<0.0002 and in females a significant age-cohort effect which, in contrast to males, consists of rising AGC incidences in birth cohorts born after 1944, p=0.01.
DISCUSSION
These results from The Netherlands confirm the world-wide trend towards rising incidence rates of oesophagus-cardia adenocarcinoma\textsuperscript{13-15}. While this rising trend was very pronounced for ACO, the AGC incidence rates actually declined for both genders. This epidemiological inhomogeneity represents the major finding of this study, indicating that, at least in The Netherlands, the rising incidence of oesophagus-cardia adenocarcinoma over the past 15 years was entirely due to ACO. Obviously, our results can be called into question on the basis of our previous criticism of the reliability of cancer registries in distinguishing between ACO and AGC\textsuperscript{1}. However, as Dutch cancer registries registered adenocarcinomas of the gastro-oesophageal junction as AGC unless Barrett’s oesophagus was mentioned, it is likely that a substantial number of cases of distal ACO were misclassified as AGC. On the other hand, it is unlikely that AGC would have been misclassified as ACO\textsuperscript{12}. Therefore, in our opinion, our ACO incidence rates actually represent a minimum. When analyzed for cohort and period effects, the annual percentage changes in ACO incidence in males were found to be linear, i.e. not significant (Table 3) However, there were obviously very considerable annual increases, amounting to over 9\% in the youngest age-cohorts (Figure 2). Therefore, in the absence of factors causing a period effect, such as a sudden change in tumour classification or the appearance of a new carcinogen and because of the significant cohort effect in females (Figure 4), we are convinced that there was in fact, a cohort effect for ACO in both genders. This outcome confirms an analysis of US SEER-data by El-Serag et al. who found a cohort effect for ACO but not for AGC\textsuperscript{19}.

We were surprised by the 2-fold gender differences in the annual percentage increases in the ACO incidence rates (Table 1). In The Netherlands, the annual percentage increase in the incidence of Barrett’s oesophagus was recently demonstrated to be equal for both genders\textsuperscript{26}. This had led us to expect equal annual percentage increases in the ACO incidence rates for both genders. However, a further analysis including the factor age, revealed the source of this large gender difference. It proved to be a precipitous “dip” in the annual percentage increases in the ACO incidence rates for females in the 61-84 age bands, i.e. born between 1905-40. As the percentage increases for the 40-85+ age group were calculated separately for each of the 15 years, this “dip” did not represent a temporary phenomenon. We suggest two mechanisms which may have been responsible for this dip, secular changes in female smoking habits and NSAID use by the elderly. Four case-control studies on the effect of smoking on the incidence of ACO and AGC combined and two of ACO and AGC separately, reported odds ratios ranging between 1.5 to 3.4\textsuperscript{27}. In The Netherlands, a study of the relative risk of lung cancer as an indicator of smoking intensity, found a steady decline in male smokers born since 1914. This in contrast to females, where smokers have steadily been increasing in successive birth cohorts since the second half of the 19\textsuperscript{th} century and, after a relative plateau between 1928-37, continued to increase, with their numbers doubling in the 1945 to ≥1958 birth cohorts\textsuperscript{28}. Therefore, although the effect of smoking on the incidence of ACO may not have been very great, the relatively low number of female smokers in the 1905-40 birth cohorts may well have contributed to the observed dip.

For NSAIDs, although there are currently no randomized trials\textsuperscript{29}, there are some basic studies\textsuperscript{30-32} and epidemiological reports\textsuperscript{33-36} suggesting a protective effect of NSAIDs against ACO in patients with Barrett’s oesophagus. In addition, there is evidence of considerable gender differences in the use of NSAIDs in the elderly\textsuperscript{37, 38}. In the Netherlands, the Integrated Primary Care Information data base which harbours the complete longitudinal electronic medical records of over 500 000 patients, revealed both a NSAID user rate, rising from 10\% below age 45 to 23\% over age45 and a 1.5-fold higher user rate in females (Dr. M.C.J.M. Sturkenboom, personal
communication). We therefore suggest that chronic NSAID use, by postponing or preventing the onset of ACO in individuals with Barrett’s oesophagus over the age of 61 and more specifically in females, because of their higher NSAID consumption, contributed to the significant reduction the annual rise in the ACO incidence rates in both genders.

The declining incidence rate of AGC was less surprising. AGC mortality trends between 1968-1994 from Netherlands already showed a decline\(^39\). However, the downward trend could also have resulted from a diagnostic shift from AGC to ACO by clinicians reporting to the cancer registries. While the presence of visible Barrett’s oesophagus was for a long time considered a condition sine qua non for the diagnosis ACO, the current trend is to look at the location of the major bulk of the tumour\(^12, 18\). Such a diagnostic shift would be a perfect explanation for the observed period effect for AGC in both genders. However, a diagnostic shift of this size should have resulted in a complementary period effect for ACO which was completely absent (Figure 3). Therefore, we believe the falling incidence rates of AGC over the past decades to represent a true phenomenon.

This raises two questions, what is AGC and which are the factors involved in its aetiology? The diagnosis AGC encompasses a complex tumour. The most commonly used Siewert classification defines AGC as an adenocarcinoma with its centre 5 cm proximal or distal from the anatomical cardia. It distinguishes 3 types, type I arising from Barrett’s oesophagus and therefore ACO, type II, true AGC, arising from the cardiac epithelium or a short segment of intestinal metaplasia at the oesophagogastric junction and type III, subcardial gastric cancer, infiltrating the cardia from below\(^17\). It should be pointed out that this is but one of a variety of hypotheses about the anatomy of the cardia\(^40\). However, several studies have suggested an intermediate epidemiological position for AGC between ACO and distal gastric cancer\(^17, 18\). It would, for instance, be conceivable that in type II AGC, gastro-oesophageal reflux plays a major role while in type III \(H. pylori\) is the dominant factor. Consequently, the incidence of AGC could reflect the algebraic sum of these two opposing aetiologies.

The currently accepted etiological factors, thought to be driving the mounting incidence of ACO in The Netherlands, are increasing obesity\(^41\) and the declining prevalence of \(H. pylori\) infection\(^42\) which mediate their effects through the induction of reflux oesophagitis and Barrett’s oesophagus\(^43\). Consequently, these factors are likely to be less relevant for AGC. For the factor obesity this expectation was confirmed in a recent meta-analysis of overweight as a risk factor for gastro-oesophageal reflux disease. In marked contrast to ACO, it found only a marginally increased risk of AGC from obesity\(^44\). Studies on the influence of \(H. pylori\) infection on AGC also failed to find significant relationships between the risk of AGC and \(H. pylori\) infection\(^45, 46\). However, another factor, smoking, may well have had a greater influence on AGC than on ACO.

Lagergren et al. in a study where by its prospective design the differentiation between ACO and AGC was particularly accurate, found AGC to be dose dependently associated with smoking, odds ratio=4.2, while the relation with ACO was weak or absent\(^47\). The contrasting secular changes in Dutch male and female smoking patterns mentioned earlier\(^28\), very nicely fit the contrasting patterns in the cohort effects observed in males and females with AGC (Figure 5) and would therefore tend to support an important role for smoking in the aetiology of AGC. In view of the 4:1 male/female ratio, the effect of the declining number of male smokers obviously considerably outweighed that of the increase in their female counterparts on the net outcome of the AGC incidence rates.

The conversion of nitrite in saliva by acid into potentially mutagenic substances such as nitrous
acid, nitrosative species and nitric oxide comprises a less well-established aetiology for oesophagus-cardia adenocarcinoma. It has been suggested that the greater use of nitrogenous fertilizers after World War II may have increased in importance of this factor\textsuperscript{48}. In patients with Barrett’s oesophagus mutagenic nitrite conversion has recently been shown to occur within the Barrett’s oesophagus segments\textsuperscript{49}. For AGC patients this conversion is likely to be localized in the cardia\textsuperscript{50}. Whether this, as yet hypothetical, factor is still on the increase or has passed its peak is unknown. However, in the absence of more solid evidence on the nitrite issue, we prefer to stay with our previous explanations for the downward trend in the incidence rates of AGC. These are the steadily falling number of male smokers and the fact that two of the major factors driving the rising incidence of ACO did not have a similar effect on the incidence of AGC.

In conclusion, this study has established the epidemiological inhomogeneity of ACO and AGC. In spite of the declining AGC incidence rates there was a 36% rise in the incidence rate of oesophagus-cardia adenocarcinoma between 1989 and 2003. This was the result of the annual growth in the ACO incidence rates of 7.2% in males and 3.5% in females. This two-fold gender difference could be attributed to a substantial fall in the annual rate of increase in elderly females, possibly linked to the relatively small number of female smokers in this age group and their higher NSAID consumption. The likely explanation for the declining AGC incidence rates was found in the combination of the precipitous fall in the number of male smokers and the observation that increasing obesity and the decreasing prevalence of \textit{H. pylori} infection, generally considered responsible for the rapidly growing ACO incidence rates, had little or no influence on the incidence of AGC.

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Chapter 7

NO RELATIONSHIP BETWEEN THE REGIONAL PREVALENCE OF HELICOBACTER PYLORI INFECTION AND THE INCIDENCE OF OESOPHAGEAL ADENOCARCINOMA

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ABSTRACT

Background: The overall incidence of adenocarcinoma of the oesophagus (ACO) is high in the Netherlands, but there are considerable regional variations. Case-control studies have suggested that colonisation with *H. pylori* provides protection against adenocarcinoma of the oesophagus.

Aims: To investigate whether a relationship could be shown between the prevalence of *H. pylori* colonisation and the incidence rates of ACO within The Netherlands

Methods: The prevalence of *H. pylori* was tested serologically in 1600 blood donors, 400 from each of four regions, per region divided into 5 equal ten-year age groups. The regions differed with respect to ACO incidence rates; two with a low incidence rate (3.4 and 3.7/10^5 in males, 0.6 and 0.9/10^5 in females), one with intermediate (4.9/10^5 in males and 1.1/10^5 in females), and one with high (6.1/10^5 in males and 1.4/10^5 in females) ACO incidence rates.

Results: There were clear age specific gradients in the prevalence of *H. pylori* colonisation; however, there were no differences in colonisation rates between the four regions.

Conclusions: This population-based study found no relationship between the contrasting incidence rates of ACO and the very constant prevalence of *H. pylori* in four regions of The Netherlands. Although not conclusive, this negative evidence calls into question the presumed protective effect of *H. pylori* colonisation against the incidence of ACO.
INTRODUCTION

The Netherlands, a country of 16 million inhabitants, has one of the highest incidences of adenocarcinomas of the oesophagus (ACO)\(^1\). Since 1989, nationwide cancer registration in this country has been carried out by nine regional registries hosted by Comprehensive Cancer Centres (CCC) working to a standard protocol. In each region all malignancies are reported to the CCC by both hospital medical record departments and pathology departments. This double case ascertainment ensures a high degree of accuracy. The Netherlands Cancer Registry in turn collates the data from all 9 CCCs\(^2\). The ascertainment of symptomatic oesophageal cancer in The Netherlands is presumed to be high, as the disease practically always results in symptoms that warrant endoscopic and histological diagnosis for curative or palliative interventions\(^3\). The country has a high incidence of adenocarcinoma of the oesophagus (ACO), yet data from the nine CCC regions have shown there to be considerable regional differences, the causes of which are unknown.

The relationship between colonisation with \textit{H. pylori} and gastro-oesophageal reflux disease has been the subject of discussion since 1997 when a negative relationship between colonisation with \textit{H. pylori} and reflux oesophagitis was first suggested\(^4,5\). Initially, in analogy with gastric carcinoma, the carcinogenic effect of the colonisation of Barrett’s oesophagus with \textit{H. pylori} was investigated. However, as this colonisation was only found in gastric type oesophageal metaplasia, no conclusions could be drawn\(^6-8\). In 1998 Richter et al. launched the hypothesis that colonisation with \textit{H. pylori} might be protective against the spectrum of gastro-oesophageal reflux disease\(^9\).

This study aimed to analyse the regional differences in the incidence rates of ACO and adenocarcinoma of the gastric cardia (AGC) and their rates of annual proportional change, and to test the hypothesis that the observed regional differences in incidence rates reflect regional differences in the prevalence of colonisation with \textit{H. pylori}.

MATERIALS AND METHODS

\textbf{Cancer data}

The annual number of cases of ACO (ICD10. C15, 3, 4 and 5) and AGC (ICD10. C16.0) for each of the 9 CCC regions was provided by the Netherlands Cancer Registry for the 15-year period 1989-2003. The localisation by CCC region was accurate as all patients were registered at their home address, irrespective of the location of the hospital in which their cancer had been diagnosed.

\textbf{Blood donors.}

In each of the four CCC regions selected for comparison, we requested the regional blood banks (Sanquin Blood Banks South-West, Rotterdam, covering CCC regions West and Rotterdam and South-East, Nijmegen, covering CCC regions South and Limburg respectively) to provide us with small aliquots of plasma from donors living in various areas within each of these 4 CCC regions. From each region, 400 samples were collected, equally divided into about 80 samples per 10-year age group between the ages of 18 and 70. The samples were anonymous, only the age and gender of the donor and the region where blood had been drawn were provided. Consequently, under Dutch law, no informed consent was required. The number of 400 in each CCC region was chosen to create a discriminative power able to identify differences of \(\geq 10\%\) in the prevalence of \textit{H. pylori} per region. Dutch blood donors are for 98\% native born Caucasians.

\textbf{Serological testing.}

Samples were centrifuged and an aliquot of supernatant plasma kept frozen at -80\(^\circ\)C until the analyses were performed. \textit{H. pylori} specific IgG antibodies were determined by a commercial ELISA kit (Pyloriset EIA-G-III, Orion Diagnostica). At a cut off titer of \(\geq 20\) positive and\(< 20\)
negative, this test achieved a sensitivity of 100 % (95% CI 95.6 - 100 %) and a specificity of 94.3 % (95% CI 88.6 - 97.7 %).

**Statistics**

Both the age standardised ACO and AGC incidence rates and their trends over the 15-year period were calculated by log-linear regression from incidence data provided by the Netherlands Cancer Registry for each of the 9 CCC regions, set out against Dutch population data for 1998. The *H. pylori* results were subjected to logistic regression. The regional prevalence rates of *H. pylori* were calculated by a model correcting for age and gender. Subsequently the increase of *H. pylori* prevalence with age was calculated by a model describing region-specific linear trends after correcting for gender. This yielded the proportional increase in the *H. pylori* colonisation prevalence for each additional year of age.

**RESULTS**

Table 1 presents the mean ACO and AGC incidence rates and the annual proportional changes in percentages for each of the 9 CCC regions over the 15-year period 1989-2003. The names of the four CCC regions selected for the comparison of *H. pylori* colonisation prevalence are underlined. The 9 CCC regions, together with their mean 15-year male ACO incidence rates, projected on a map of the Netherlands, are shown in Figure 1. These mean incidence rates varied between 3.4/10^5 in CCC region Limburg and 6.3/10^5 in CCC region North. The differences between the various region were statistically significant. The highest mean ACO incidence rates can be seen to be found in the north-eastern and south-western regions, with intermediate values in a large part of the country lying between these two regions. The lowest mean incidence rates were localised in the south-eastern region, extending into the central part of the country. For females the mean ACO incidence rates followed very similar patterns although at very much lower levels (Table 1). Besides these regional variations in ACO incidence rates, there were also wide variations in the regional proportional changes which in males ranged between 4.6 to 4.7% in Limburg and the Amsterdam CCC region to 13.8% in the South CCC region. Both the ACO incidence rates over the 15-year period and the trends in the proportional changes for the four CCC regions involved in the comparison of *H. pylori* colonisation prevalence are illustrated in Figure 2. Fairly steep parallel trends in ACO incidence rates, although at different levels, were seen in the Rotterdam and South regions, as against far less steep trends, again at different levels, in the West and Limburg regions. These differences were all significant.

The spread in the AGC incidence rates was far smaller than in ACO and here there was a tendency towards stabilisation or decline, the latter significantly in CCC regions South, Middle and Rotterdam (Table 1).

The prevalence of *H. pylori* serum antibodies in the four compared CCC regions, presented by age and gender, are shown in Table 2 and Figure 3. The statistical analyses of the prevalence of *H. pylori* per CCC region and the increases of this prevalence for every extra year of age are presented in Table 3. After correction for age and gender there were no statistically significant differences in the prevalence of *H. pylori* between the four CCC regions, p=0.87. In addition there were no significant differences between males and females. The proportional increase of the *H. pylori* prevalence for each extra year of age was almost identical for all regions, with the exception of CCC West. However, the difference between the latter region and the other three was not statistically significant, p=0.14 (Table 3). Consequently, in these four CCC regions, chosen for comparison, there were neither parallels between the widely varying regional incidence rates of ACO and the identical prevalence of *H. pylori* colonisation, nor between the regional annual proportional trends in the rising incidence rates.
of ACO and the again identical age-related proportional changes in the prevalence rates of *H. pylori* in these four regions.

Table 1. Mean ACO and AGC Incidence Rates and their Annual Proportional Changes per CCC Region

<table>
<thead>
<tr>
<th>CCC Region</th>
<th>Mean Incidence Rates/10^5</th>
<th>Annual % Change</th>
<th>Mean Incidence Rates/10^5</th>
<th>Annual % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Limburg</td>
<td>3.4</td>
<td>0.6</td>
<td>4.6*</td>
<td>5.7</td>
</tr>
<tr>
<td>95% CI</td>
<td>2.9-3.9</td>
<td>0.5-0.8</td>
<td>3.1-4.0</td>
<td>0.8-1.3</td>
</tr>
<tr>
<td>South</td>
<td>3.7</td>
<td>0.9</td>
<td>13.8**</td>
<td>9.0**</td>
</tr>
<tr>
<td>95% CI</td>
<td>3.4-4.1</td>
<td>0.8-1.0</td>
<td>4.3-5.2</td>
<td>1.1-1.4</td>
</tr>
<tr>
<td>Middle</td>
<td>3.8</td>
<td>1.1</td>
<td>6.5**</td>
<td>2.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>3.3-4.2</td>
<td>0.9-1.3</td>
<td>4.6-5.6</td>
<td>1.0-1.4</td>
</tr>
<tr>
<td>East</td>
<td>4.0</td>
<td>0.8</td>
<td>10.5**</td>
<td>7.2*</td>
</tr>
<tr>
<td>95% CI</td>
<td>3.6-4.5</td>
<td>0.7-1.0</td>
<td>3.8-4.7</td>
<td>0.6-0.9</td>
</tr>
<tr>
<td>West</td>
<td>4.9</td>
<td>1.1</td>
<td>5.4**</td>
<td>2.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>4.5-1.3</td>
<td>1.0-1.3</td>
<td>4.6-5.5</td>
<td>0.9-1.3</td>
</tr>
<tr>
<td>Amsterdam</td>
<td>5.3</td>
<td>1.3</td>
<td>4.7**</td>
<td>1.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>5.0-5.7</td>
<td>1.2-1.5</td>
<td>4.6-5.3</td>
<td>0.9-1.2</td>
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<tr>
<td>Twente</td>
<td>5.4</td>
<td>1.4</td>
<td>5.5**</td>
<td>-0.4</td>
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<tr>
<td>95% CI</td>
<td>4.9-5.9</td>
<td>1.2-1.7</td>
<td>3.9-4.8</td>
<td>0.0-1.7</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>6.1</td>
<td>1.4</td>
<td>8.2**</td>
<td>3.2x</td>
</tr>
<tr>
<td>95% CI</td>
<td>5.7-6.5</td>
<td>1.3-1.6</td>
<td>4.8-5.5</td>
<td>1.0-1.3</td>
</tr>
<tr>
<td>North</td>
<td>6.3</td>
<td>1.3</td>
<td>7.5**</td>
<td>6.2**</td>
</tr>
<tr>
<td>95% CI</td>
<td>5.9-6.7</td>
<td>1.2-1.5</td>
<td>4.9-5.6</td>
<td>0.8-1.1</td>
</tr>
</tbody>
</table>

Significance of Annual % Change: x p= 0.01-0.05, * p=0.005-0.01, ** p<0.0001

Table 1 presents the age standardised incidence rates of adenocarcinoma of the oesophagus (ACO) and of the cardia (AGC) calculated by log-linear regression for the period 1989-2003 for both genders in the 9 Comprehensive Cancer Centre regions of The Netherlands. In addition, the annual percentage change in these incidence rates and their statistical significance are presented.
Table 2 presents the results of the *H. pylori* serology in the four compared Comprehensive Cancer Centre (CCC) regions. South and Limburg are low adenocarcinoma of the oesophagus incidence regions, West a medium and Rotterdam a high incidence region. There are no significant differences between the 4 regions in the prevalence of *H. pylori*, p=0.87. N= number of tested donors; Hp= number of donors with positive *H. pylori* serology; % = percentage of tested donors with positive *H. pylori* serology.

Table 3 presents the odds ratios for the levels of the *H. pylori* prevalence in the four Comprehensive Cancer Centre regions set against the mean value for the 4 regions of 1. There are no significant differences, p=0.87. The proportional increase for each additional year of age is practically the same for all 4 regions, with the exception of West, but this difference is not significant, p= 0.14.
Figure 1

A map of The Netherlands showing the 9 Comprehensive Cancer Centre (CCC) regions. The grey scale provides an indication of the mean adenocarcinoma of the oesophagus incidence rates for males over the period 1989-2003 in each region, the actual mean incidence rates are shown in each CCC region. Note the lower incidence rates in the South-Eastern regions, extending into the central area and the high incidence rates in the North-East and South-West.

Figure 2

shows the trends in the incidence rates of adenocarcinoma of the oesophagus (ACO) from 1989-2003 for the four CCC regions involved in the H. pylori prevalence comparison. Rotterdam and South show similar, high annual increases at different levels, while West and Limburg share slower growth, again at different levels. In females both overall rates and annual growth are far lower but similar in pattern.
Figure 3. The percentage of male and female *H. pylori* serologically positive blood donors found in the 4 Comprehensive Cancer Centre regions. Note that for the total 18-70 year age group the results for the 4 regions are practically identical.

**DISCUSSION.**

This study shows that there are up to almost two-fold differences in the incidence rates of ACO in various parts of The Netherlands. At first sight this was a rather surprising finding as it involves a small country with a high standard of living and relatively small differences between social classes. The only general factor distinguishing the high and low ACO incidence rates was that the former were situated in the formerly predominantly Protestant provinces bordering the North Sea and the latter in the inland, formerly predominantly Roman Catholic provinces of the South-East. Until the middle of the 20th century the South-East, South-West and North-East were less affluent, but these differences have practically disappeared over the last 40 years. Nevertheless, only 25 years ago predominant Roman Catholic regions were still characterised by excess mortality figures in comparison with other parts of the country. This was mainly attributed to a higher prevalence of smoking. Although these differences were far smaller than those currently found in ACO and had been declining since 1950, these data provided evidence for the influence of the variations in lifestyle within our small country on regional health outcomes.

Could these different ACO incidence rates be artefacts resulting from differing cancer registration policies? All 9 CCCs function according to a centralised protocol and there are regular meetings intended to coordinate practices. In addition, there are close national contacts between gastroenterologists, pathologists and surgeons involved in treatment of these patients, among others guided by national guidelines. Together, this makes it unlikely that the observed differences can be explained by registration policies. In addition, Table 1 shows that the regional differences for AGC were far smaller than for ACO and for squamous cell carcinoma of the oesophagus the differences were even smaller (data not shown).

One of the factors which has been cited as a cause for the rapidly rising incidence of Barrett’s oesophagus and consequently, of ACO in Western industrialised countries, is the declining prevalence of colonisation with *H. pylori*. The observation that Eastern European and
Asian countries, with far higher colonisation rates of *H. pylori* than in the West, have considerably lower rates of Barrett’s oesophagus and ACO supports this concept. However, the comparison between geographically and culturally very diverse countries obviously introduces many potential confounding factors. Currently there is only one published population based study of the relationship between the prevalence of *H. pylori* colonisation and the ACO incidence rates within a single country. Here the controls consisted of age and gender matched persons randomly selected from the Swedish population register. We decided to approach our population based study by estimating the prevalence of *H. pylori* colonisation in healthy inhabitants (blood donors) in four Dutch CCC regions with relatively low and high incidence rates of ACO in order to establish whether a link could be demonstrated between the *H. pylori* prevalence in the general population of these four regions and their varying ACO incidence rates. The results of the *H. pylori* testing demonstrated a significant age specific gradient in the *H. pylori* prevalence (Table 3), an outcome compatible with a birth cohort effect in *H. pylori* prevalence which has been found in several European countries. However, there were no indications of any relationship between the regional *H. pylori* prevalence and the incidence rates of ACO. The size of the tested groups of blood donors was adequate as shown by the confidence intervals of the *H. pylori* prevalence data, which were related to the size of the sample tested in each CCC region (Table 3). The choice of blood donors as representing the whole population could be criticised on the grounds that they are for over 98% recruited from the native Dutch population, while currently about 10% of the population consists of firsts and second generation immigrants, in particular from Mediterranean, South American and Caribbean origin in whom the *H. pylori* colonisation rates are far higher than in the native Dutch population. However, perusal of the records of the 1088 patients presented to the Rotterdam Oesophageal Cancer Group over the 10-year period 1994-2003 revealed only 29 patients born outside The Netherlands (data not published). Of these, only two patients with ACO came from Mediterranean countries and two from South East Asia. This suggests that, in The Netherlands, ACO is currently still predominantly a disease of the native Dutch population and therefore the colonisation prevalence of *H. pylori* as found in blood donors is relevant for the epidemiology of ACO, a statement which would obviously not be true for the epidemiology of peptic ulcer or gastric cancer. Another potential confounding factor could result from regional variations in the number of donors who had undergone *H. pylori* eradication. However, a general practitioner electronic data bank, covering 500 000 patients from all over the country, revealed that the number of *H. pylori* eradication treatments had ranged between about 5/10 000 patient years in the 40-year age group to about 18/10 000 patient years in the 60- year age group (E. van Soest, data not published), i.e. over the previous 10 years, out of our 1600 tested donors, one or two might have undergone *H. pylori* eradication. Our negative results do not come as a complete surprise. The relationship between *H. pylori* infection and reflux oesophagitis and its sequelae has been questioned previously. Vieth et al. claimed to have established this relationship on the basis of the retrospective analysis of gastric and oesophageal biopsies obtained from a total of 2,201 patients. They found 297 patients with gastro-oesophageal reflux disease, 1054 with Barrett’s oesophagus and 138 with Barrett’s neoplasia (high-grade dysplasia or adenocarcinoma). A total of 712 patients with non-ulcer dyspepsia served as a control group. There were no significant differences in *H. pylori* colonisation between patients with gastro-oesophageal reflux disease (51.4%) Barrett’s oesophagus (53.3%) and Barrett’s neoplasia (47.8%). While these percentages were significantly lower than in controls, the *H. pylori* prevalence of 65.7% in this control group was unacceptably high for a Western European population and does not
admit the conclusion that there were significant differences between patients and the general population.\(^23\)

Finally, our study provides renewed data on the age specific and overall prevalence of *H. pylori* serum antibodies in our Western population. This prevalence in blood donors was 35% overall in 1989\(^24\) and is currently, in spite of the age cohort effect, still 32% indicating that over the foreseeable future *H. pylori*-related diseases are likely to remain prevalent in The Netherlands, not only in immigrants but also in the native Dutch population. In conclusion, this study investigating the relationship between the prevalence of *H. pylori* colonisation and the strongly differing incidence rates of ACO in various regions of The Netherlands, failed to find the expected inverse relationship between these two factors. This lack of positive evidence calls into question the presumed protective effect of *H. pylori* infection against the incidence of ACO. In addition, it clearly indicates that other factors must be sought to explain the observed regional differences in ACO incidence rates and their proportional changes within this country. In view of the steep rise in the incidence of ACO and its precursors in Western countries\(^25-27\) research is needed to reveal the factors responsible for this rise, which may in the near future also occur in other parts of the world such as Asia\(^28-30\).

Acknowledgment: this study was made possible by a grant from the Gastrostart Foundation.

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Chapter 8

DISCUSSION, CONCLUSIONS AND SUMMARIES
DISCUSSION

Chapter 1. Introduction.

The definition limiting Barrett’s oesophagus to metaplastic cylinder cell lined oesophageal mucosa containing specialised intestinal metaplasia, is criticised on the grounds that two forms of cylinder cell lined oesophagus, one innocent and one progressing to cancer, are both implausible and not confirmed by any clinical follow up studies. In fact, both forms often co-exist¹ and a British follow up study found equal numbers of adenocarcinomas of the oesophagus in patients with and without specialised intestinal metaplasia at their first endoscopy².

The historical section is chiefly of interest to the historically minded. However, the section on the history of experimental reflux oesophagitis describes studies on the effects of acid, bile acids and pancreatic enzymes on the oesophageal mucosa which are still relevant today. In addition, the efforts of the pioneers in the analysis of the motility disturbances underlying reflux oesophagitis and ultimately, Barrett’s oesophagus, deserve to be recorded.

Unfortunately, the success of proton pump inhibitors in treating reflux oesophagitis appears to have dimmed the interest in its pathophysiology. Although techniques such as ambulatory 24-hour oesophageal pH, bilirubin and motility monitoring and oesophageal impedance measurements of gas and non-acid fluids have added much to our insights into the when and where of duodeno-gastric-oesophageal reflux, little more is known about the what, i.e. which bile acids or enzymes in what concentrations are present where at any given time of day. The relevance of such data is shown by the very considerable overlap between the degree of reflux, as measured by pH monitoring and the severity of oesophagitis or presence of Barrett’s oesophagus. Although genetical factors may also be at work, it is likely that the composition of the refluxate plays an important role in triggering both inflammation and carcinogenesis. This lack of clinical interest in pathophysiology stands in stark contrast to the impressive developments in the fields of molecular biology. The insights into the steps leading to malignancy and the results of genetic profiling, of which some examples are discussed, may eventually lead us to the Holy Grail, the ability to effectively predict the impending onset of adenocarcinoma from biopsies of Barrett’s oesophagus.

Chapter 2. The Incidence of Adenocarcinoma in Barrett’s Oesophagus in an Institutionalized Population.

Eur J Gastroenterol Hepatol 2004;16: 903-909

This study combined data from 2 studies previously published by Dr. Clarisse Böhmer who had studied the prevalence of gastro-oesophageal reflux disease by 24-hour pH monitoring in an age and gender representative randomly selected group of inhabitants of institutions for the severely mentally handicapped, here called IDI.

As expected by the investigator, the prevalence of both gastro-oesophageal reflux disease and of gastro-oesophageal reflux oesophagitis and Barrett’s oesophagus, for reasons as yet unknown, was extremely high in these IDI³. The second study observed the number of cases oesophageal cancer, and specifically that of adenocarcinoma of the oesophagus, in these institutions over a period of six years⁴. The current study, by extrapolating the observed prevalence of Barrett’s oesophagus in the randomly selected representative group of IDI, estimate of the size of the total IDI population with Barrett’s oesophagus. From this data and the observed adenocarcinoma of the oesophagus the incidence rates of adenocarcinoma of the
oesophagus calculated for this population with Barrett’s oesophagus. In spite of the relatively small IDI population and therefore the limited number of IDI follow up years, the about tenfold prevalence of Barrett’s oesophagus did enable the estimation of relevant adenocarcinoma of the oesophagus incidence rates which, after correction for age distribution, proved to be similar to the adenocarcinoma of the oesophagus incidence rates from the Rotterdam Barrett’s oesophagus follow up study group of free living individuals with full access to alcohol and tobacco.

The study includes several interesting points. Neither the prevalence of gastro-oesophageal reflux disease and oesophagitis nor that of Barrett’s oesophagus had ever been surveyed in randomly selected patients not expressing any symptoms. Because the IDI do not smoke or use alcohol and this fact is guaranteed by their institutionalization, the finding of the “usual” 10% prevalence of Barrett’s oesophagus in the IDI with gastro-oesophageal reflux disease, would tend to exonerate alcohol and tobacco, both from the aetiology of Barrett’s oesophagus and that of adenocarcinoma of the oesophagus. The differentiation between adenocarcinoma of the oesophagus and of the cardia is always a problem. Involvement of the cardia was reported in 4 of 18 adenocarcinomas, however, Barrett’s oesophagus was found in 9 and reflux oesophagitis in 14, so that while the number of adenocarcinomas of the oesophagus was presumably only 14, it is notable that a ratio of 14:4 for adenocarcinoma of the oesophagus to adenocarcinoma of the cardia is remarkably different from the usual 50:50 ratio, certainly in this period. This observation could support the idea that smoking does play a role in the aetiology of adenocarcinoma of the cardia.

Chapter 3. Age and sex distribution of the prevalence of Barrett's oesophagus found in a primary referral endoscopy center.


This study again made use of data published by others, in this case the number of cases of Barrett’s oesophagus diagnosed over a 15-year period in patients predominantly referred for endoscopy by general practitioners. By adding in the number of first endoscopies performed in each of three five-year periods as a denominator, it was possible to calculate the percentage of patients with Barrett’s oesophagus, including those with and without specialised intestinal metaplasia, by age and sex for each 5-year period and the full 15 years. Further statistical analysis revealed that for Barrett’s oesophagus in general and both histological subtypes, the percentage increase in prevalence for each additional year of age was constant in both genders, for males until 60 and females until 80 years of age. In addition, a 20-year shift in the age of onset of Barrett’s oesophagus between men and women was demonstrated. This finding, including the 20-year age shift, was recently confirmed in a study of data from an electronic general practitioner’s databank in The Netherlands. This 20-year age shift, if confirmed in further studies, is an extremely important finding. In the first place it goes a long way to explain the 2:1 gender ratio in Barrett’s oesophagus and the even higher gender ratio in adenocarcinoma of the oesophagus. The age shift is the result of low incidence rates of Barrett’s oesophagus in young, fertile women and obviously suggests hormonal influences which would be interesting to explore. The relationship between the prevalence of gastro-oesophageal reflux oesophagitis and overweight in women could suggest that this hypothetical hormonal protection for women may disappear with their tendency to gain weight around the age of 40. The identical rates of increase in the prevalence of Barrett’s oesophagus with age provided further arguments against the concept of two types of Barrett’s oesophagus, with and without specialised intestinal metaplasia.
Chapter 4. The Incidence of Adenocarcinoma and Squamous Cell Carcinoma of the Oesophagus; Barrett's Oesophagus makes a Difference.


This study again made use of previously published data, this time on the incidence of oesophageal cancer, and specifically adenocarcinoma of the oesophagus, in Denmark. A strong point of this data was that the distinction between adenocarcinoma of the oesophagus and adenocarcinoma of the cardia had been revised by a panel of gastroenterologists, using original clinical data. After adding population data, age and gender specific incidence rates were calculated for adenocarcinoma of the oesophagus, adenocarcinoma of the cardia and squamous cell carcinoma of the oesophagus.

The most interesting finding was a declining incidence rate of adenocarcinoma of the oesophagus in the oldest age group. The fact that this was not caused by under diagnosis was demonstrated by the absence of a similar decline in the incidence rate squamous cell carcinoma of the oesophagus. This finding posed the question whether this decline was the result of a birth cohort effect, a question which could not be answered as it was impossible to exclude the possibilities of it being caused by the extra mortality from adenocarcinoma of the oesophagus, to which the population with Barrett’s oesophagus is prone or from a hypothetical inferior state of health of this population.

Chapter 5. Modelling a Population with Barrett’s Oesophagus from Oesophageal Adenocarcinoma Incidence Data


This study forms a companion to that in chapter 4. The finding that the great majority of persons with Barrett’s oesophagus are never diagnosed, results in practically all data on the prevalence of Barrett’s oesophagus being derived from the small minority who are ascertained by clinical endoscopy, mostly performed for reflux symptoms. The population with adenocarcinoma of the oesophagus is, however, selected for a completely different symptom and consequently, is likely to reflect the composition of the largely hidden Barrett population far more accurately than data provided by clinical endoscopy.

By using Danish adenocarcinoma of the oesophagus incidence rates and a complicated statistical technique it was possible to choose between the three hypotheses explaining the declining incidence rates of adenocarcinoma of the oesophagus in elderly Danes, resulting in a choice for the birth cohort phenomenon. In addition, it was possible to calculate the age specific incidence rates of adenocarcinoma of the oesophagus for a hypothetical cohort of persons who had acquired Barrett’s oesophagus before the age of 40. However, the limitations of this model meant that it was impossible to extend these incidence rates to the whole Danish population with Barrett’s oesophagus. To achieve this goal a new model will have to be designed, which will need to encompass the growth of the population with Barrett’s oesophagus on the basis of the findings in chapter 3. Simplistic calculations, unsupported by any statistician, suggest that this growth tends to neutralize the age specific increase in the incidence of adenocarcinoma of the oesophagus, resulting in a fairly constant incidence rate of adenocarcinoma of the oesophagus in persons with Barrett’s oesophagus, which is in accordance with clinical observations.

submitted for publication

This study of the incidence rates of adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia examined the question whether over the past 15 years the incidence rates of both cancers have been increasing at the same rate. The inclusion of age-period-cohort analyses both widened the scope of the analyses and guarded against the occurrence of diagnostic shift from adenocarcinoma of the gastric cardia to adenocarcinoma of the oesophagus. An unequivocal rise in the incidence rates of adenocarcinoma of the oesophagus and a somewhat smaller fall in those of adenocarcinoma of the gastric cardia were observed. The fact that a clear period effect in the falling incidence rates of adenocarcinoma of the gastric cardia was not mirrored by a similar period effect in the rise of the incidence rates of adenocarcinoma of the oesophagus made a diagnostic shift from adenocarcinoma of the gastric cardia to adenocarcinoma of the oesophagus most unlikely. In adenocarcinoma of the oesophagus the age specific percentage rise in incidence rates of women between the ages of 60 and 84 declined dramatically in comparison with males. It was suggested that this decline could have been the result of the higher consumption of NSAIDs in elderly females. These declining rates of increase of the adenocarcinoma of the oesophagus incidence may also have contributed to the 4:1 gender ratio in the incidence rates of adenocarcinoma of the oesophagus; a more intriguing question is whether we can observe the effect of the 20-year age shift in the onset of Barrett’s oesophagus. Numerically, a comparison between the number of cases of adenocarcinoma of the oesophagus in men between the ages of 25 and 69 and women between the ages of 45 and 85+ would tend to explain the larger part of the 4:1 gender ratio. However, this hypothesis has not yet been blessed by statistical approval. It is possible to conclude that the steeply rising incidence rates of adenocarcinoma of the oesophagus were the cause of the rising incidence rates of oesophageal adenocarcinoma in the 15 years between 1989 and 2003. The incidence rates of adenocarcinoma of the gastric cardia actually declined, possibly as the result of the falling number of male smokers.

Chapter 7. No Relationship between the Regional Prevalence of Helicobacter pylori Infection and Adenocarcinoma of the Oesophagus

To be submitted for publication

The large differences in the incidence rates of adenocarcinoma of the oesophagus in males and to a lesser extent in females, found in the 9 Comprehensive Cancer Centre regions, offered a challenge to explore the aetiological factors involved. There is a fairly extensive literature, mainly based on case control studies, that infection with Helicobacter pylori provides protection against the incidence of Barrett’s oesophagus and adenocarcinoma of the oesophagus. This hypothesis was addressed in this study by measuring the prevalence of Helicobacter pylori infection in 4 Comprehensive Cancer Centre regions selected for their divergent incidence rates of adenocarcinoma of the oesophagus. The prevalence of Helicobacter pylori infection was examined in about 400 blood donors from each of these 4 regions. To our surprise there were no significant differences between the prevalence of Helicobacter pylori infection in the 4 regions and therefore no relationship with the very significant differences in the incidence rates of adenocarcinoma of the oesophagus. This study therefore calls into question the protective effect of Helicobacter pylori infection against
adenocarcinoma of the oesophagus. Possibly further studies could be envisaged comparing the prevalence of *Helicobacter pylori* in patients with Barrett’s oesophagus and adenocarcinoma of the oesophagus with the *Helicobacter pylori* prevalence in their region.

**CONCLUSIONS**

The observations described in this thesis on the epidemiology of Barrett’s oesophagus and its final stage, adenocarcinoma of the oesophagus, were not part of a preconceived research plan but were inspired by a desire to learn more about the epidemiology of these conditions. In researching background information for chapter 2 “the incidence of adenocarcinoma in Barrett’s oesophagus in an institutionalised population” it became clear that epidemiology was a rather neglected field in the otherwise richly cultivated landscape of the literature on Barrett’s oesophagus, with however, all the opportunities which such relatively uncultivated fields afford.

The problem of the mainly hidden population of Barrett’s oesophagus was now approached from two directions. The first was the estimation of the prevalence of Barrett’s oesophagus within a large number of patients referred by general practitioners for a wide variety of indications in order to avoid patient selection which inevitably occurs in larger centres. By converting the originally published Barrett’s oesophagus prevalence data into prevalence rates per 100 first endoscopies it became possible to discover a certain systematic order from these data. It revealed that in both genders the prevalence of Barrett’s oesophagus increased by a fixed percentage for each additional year of age, be it that in women this increase was delayed by 20 years and this delay, or age shift, was responsible for the 2:1 male to female ratio in the prevalence of Barrett’s oesophagus. It was a great relief when a study by Eva van Soest, using data from a Dutch electronic general practitioners data base, confirmed this finding. The causes of this 20 year period of grace granted to women before acquiring Barrett’s oesophagus should be an obvious field for further research.

The second approach was through adenocarcinoma of the oesophagus which forms the final chapter in Barrett’s oesophagus. This tumour has a fairly high ascertainment rate and, in contrast to Barrett’s oesophagus, there is no selection bias towards symptoms of reflux oesophagitis, in practice there are fairly uniform symptoms and investigations leading to diagnosis. By converting Danish adenocarcinoma of the oesophagus incidence data to incidence rates and employing further statistical techniques it was possible to demonstrate a birth cohort effect in the rising incidence rates of adenocarcinoma of the oesophagus in Denmark and fitting to the observed rises after the observation period. A birth cohort effect implies that the factors responsible for the rising incidence rates of adenocarcinoma of the oesophagus are probably already operational in a large part of the population. This means that uncovering these factors is of vital importance if any form of primary prevention is envisaged. A current front runner would appear to be obesity which appears responsible for an increasing prevalence and severity of gastro-oesophageal reflux disease. In addition the mathematical modeling has provided a first step in estimating the total population with Barrett’s oesophagus which is for over 90% undetected. Such estimates would be valuable in planning secondary prevention.

The data on the incidence rates of adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia, provided by the Association of Comprehensive Cancer Centres and collated by the Netherlands Cancer Registry, proved another valuable source. Analysis of these data, (see chapter 6) has shown that in The Netherlands the incidence rates of adenocarcinoma of the gastric cardia are in decline while those of adenocarcinoma of the oesophagus are rising
rapidly. Although some speculations are advanced in chapter 6 to explain these different trends, some serious efforts should be made to provide more scientifically based explanations. Another interesting finding were the major differences in the incidence rates of adenocarcinoma of the oesophagus in various regions of The Netherlands, differences which were far smaller for adenocarcinoma of the gastric cardia. These regional variations are again an obvious target for further analysis. A first attempt, a comparison between the *Helicobacter pylori* colonisation rates in blood donors from regions with low and high incidence rates of adenocarcinoma of the oesophagus failed to reveal any differences.

Finally, having established that the incidence of Barrett’s oesophagus and consequently that of adenocarcinoma of the oesophagus is rising rapidly in Western industrialised countries and this rise is likely to continue, this raises the question: what can be done? In the first place a sense of proportion is essential. A number of studies initiated in Rotterdam by Jan Dees have shown that only a small percentage of people with Barrett’s oesophagus actually die from adenocarcinoma of the oesophagus. Although both the diagnostic and therapeutic potentials of endoscopic surveillance of patients with Barrett’s oesophagus have undergone revolutionary changes, the main problem remains that the ascertainment of Barrett’s oesophagus is less than 10% of the population with this condition. Endoscopic screening of all males over the age of 50 would not appear to lie within the realm of reality while the public acceptance is unlikely to be high. The answer must probably be sought in the field of genomics and/or proteomics which can potentially provide simple blood tests to identify individuals with Barrett’s oesophagus and for their subsequent treatment.
SUMMARIES

Chapter 1.

Barrett’s oesophagus is defined and the history of the gradually emerging relationship between reflux oesophagitis, Barrett’s oesophagus, and adenocarcinoma of the oesophagus, is sketched in the historical section. This leads on to the attempts made over several decades to unravel the pathophysiology of reflux oesophagitis. A brief overview is presented of the clinical tests and classic balloon and manometric studies which revealed the importance of the failure of lower oesophageal sphincter function and of oesophageal motility in the aetiology of reflux oesophagitis. The experimental studies on the effects of various substances in the refluxate on the oesophageal mucosa are described in some detail as their outcome are still relevant today. In the present state a brief outline is given of the modern insights into the genetic changes occurring during the progress from reflux oesophagitis to Barrett’s oesophagus and adenocarcinoma of the oesophagus. The current thinking on the aetiology of reflux oesophagitis are discussed in the light of the effects of strong acid inhibition by proton pump inhibitors.

Chapter 2.

A study designed to ascertain the incidence rate of adenocarcinoma in Barrett’s oesophagus (ACO) in a stable population of 28,000 institutionalised intellectually disabled individuals (IDI) in whom the prevalence rate of Barrett’s oesophagus (BO) was previously estimated in a representative sample by 24-hr pH monitoring and endoscopy. In this population all cases of ACO were ascertained over a 6 year period. These IDI do not smoke or drink alcohol and were known to have exceptionally high prevalence rates of gastro-oesophageal reflux disease (GORD) and consequently of BO. The six year observation of the IDI population comprised 52,038 person years, within this period all cases of cancer of the oesophagus were ascertained and the histological diagnosis established from medical records. On the basis of the representative sample, the percentage of this population with BO was estimated to be 10.8%. ACO incidence rates could then be estimated and compared to those found in a free living population with BO, after correction for age and gender. In IDI an incidence rate of ACO of 2.5/1000 person years was found against 6.3/1000 person years in the free living population. However, the age distributions of the IDI and of the free living population were very different, and after correction for this factor there was no significantly lower incidence rate of ACO in the IDI (relative risk: 0.79; p= 0.61). This is the first reported incidence study of ACO in a stable, well defined population in which the ascertainment of BO was not based on endoscopy for symptoms but on random selection. In contrast to squamous cell carcinoma, the similar ACO incidence rate of this abstemious population to that of a free living population suggest only a minor role for smoking and alcohol in the aetiology of ACO.
Chapter 3.

Both the demographics underlying the sex ratio in the prevalence of Barrett’s oesophagus (BO) and the status of BO without intestinal metaplasia (IM) are unclear. This study set out to establish the demographics of histologically proven BO, both IM+ and IM-, as observed over a 15 year period at a primary referral, endoscopy unit. For all BO patients aged 20-89 and identified between 1982-96, BO IM+ or IM-, prevalence rates per 100 first endoscopies were calculated. 492 cases of BO, 320 (248 IM+) in males, 172 (127 IM+) in females were identified in 21,899 first endoscopies (10,939 males, 10,960 females). Between ages 20-59 in males and 20-79 in females, both IM+, IM- BO and all BO prevalence rates rose by ±7.36% for each additional year of age (p=0.92) with however, a 20 year age shift between the sexes, resulting in a male/female O.R. 4.15, 95%CI 2.99-5.77. A declining rate of increase in over 59 males finally resulted in an overall male/female O.R. 2.14, 95%CI. 1.77-2.58. Over the age of 79, BO prevalence rates/100 first endoscopies fell from a maximum of 5.1 in males and 3.65 in females to 3.38 and 2.53 respectively. We concluded that the 4:1 sex ratio and 20 year age shift between males and females in the prevalence of BO, both IM+ and IM- BO, found in younger age groups, was the main cause of the overall BO 2:1 sex ratio. The very similar demographics of IM- and IM+ BO suggest they may well be 2 consecutive stages in the same metaplastic process.

Chapter 4.

Adenocarcinoma limited to the oesophagus (ACO) arises in Barrett’s oesophagus (BO). The incidence of ACO is therefore restricted to this BO sub-population, whose size is unknown and which is for 95% unidentified. We set out to determine the age and gender specific incidence rates of ACO, limited to the BO sub-population, within a defined geographical area and to compare them with those of squamous cell carcinoma of the oesophagus (SCC), which can affect the entire population. The age and gender specific incidence rates for ACO and adenocarcinoma of the cardia (AGC) were calculated after an expert panel classified 87% of all cases of adenocarcinoma of the oesophagus, reported to the Danish Cancer Registry over a six year period, as ACO or AGC. The age specific incidence rates of ACO for males rose from 0.09/10^5 (30-34 yr) to 14.14/10^5 (80-84 yr), falling to 7.2/10^5 (85+ yr), for females from 0.19/10^5 (45-49 yr) to 2.79/10^5 (80-84 yr), falling to 2.43/10^5 (85+ yr) and yielding a gender ratio of 5.9:1; AGC demonstrated a similar pattern and a gender ratio of 4.26:1. However, the incidence rates of SCC continued rising after age-80, with a gender ratio of 2.5:1. The continuing rise in the SCC incidence rates in the elderly demonstrated that the unexpected decline and fall in the incidence rates of ACO over age-80 did not result from under diagnosis but were most probably caused by a declining prevalence rate of BO, restricting the elderly BO sub-population at risk of developing ACO. The difference between the 6:1 gender ratio in ACO and 2.5:1 in SCC was ascribed to the 2:1 or greater gender ratio in BO.
Chapter 5.

The study described in the previous chapter, analysing the adenocarcinoma of the oesophagus (ACO) incidence rates in Denmark, revealed a steep fall in the over-80 population, interpreted as the result of a declining prevalence of Barrett’s oesophagus (BO) in this age group for which three hypotheses were advanced: the specific mortality from ACO and, superimposed, either excess mortality from causes of death unrelated to ACO or a birth cohort effect.

On the basis of the observed ACO incidence rates, we attempted to create statistical models estimating the BO population fitting each of these 3 hypotheses and, by selecting the most plausible hypothesis, to gain insight into the composition of the Danish BO population. The models which were designed for three hypotheses conformed to the generally accepted 0.4-0.5% annual ACO incidence in BO patients. These models employed expectation-maximization algorithms, Danish life tables and the observed ACO incidence rates. The models enabled the estimation of a BO population for each hypothesis.

After testing against previously set criteria, the most plausible model was found to be that describing a birth cohort effect, which was found to predict a ± 5% annual rise in the prevalence of BO and consequently, in the incidence rate of ACO in Denmark. This prediction was borne out over the decade following on the ACO observation period. This rising ACO incidence rate is likely to continue into the foreseeable future. The use of EM algorithms enabled a first estimate of the BO population at risk of ACO although, owing to the limitations imposed by the models, the age and gender specific ACO risk for the entire Danish BO population could not as yet be ascertained, this will require a more complicated model.

Chapter 6.

In common with other industrialized countries, The Netherlands has experienced a rising incidence of oesophagus-cardia adenocarcinoma, raising the question of the respective roles of the two constituent tumours, i.e., adenocarcinoma of the oesophagus (ACO) and adenocarcinoma of the gastric cardia (AGC) in this rise.

We set out to answer this question by comparing time trends for the incidence of ACO and AGC in The Netherlands while also controlling for registration artefacts. The trends in the 1989 to 2003 annual incidence rates by 3-year age classes and gender for ACO and AGC, as provided by the Netherlands Cancer Registry, were compared. In addition, these data were analyzed for age-period-cohort patterns which also provided a check on diagnostic drift from AGC to ACO. Over the 15-year period, the annual rise in the incidence rate of oesophagus-cardia adenocarcinoma was 2.6% for males and 1.2% for females. This was the net outcome of annual increases in ACO incidence (7.2% for males and 3.5% for females) and annual rate of decline in AGC incidence of over 1% for both genders. For ACO and AGC, both genders exhibited a non-linear cohort pattern, whereas a non-linear period pattern was only observed for males with AGC.

We concluded that the divergent time trends and age, period and cohort patterns observed between ACO and AGC clearly establish their epidemiological heterogeneity. The declining AGC incidence probably resulted from both the secular decline in the number of male smokers and that the two main aetiologial factors, considered to be driving the rising ACO incidence, do not affect the incidence of AGC.
Chapter 7

The overall incidence of adenocarcinoma of the oesophagus is high in the Netherlands, but there are considerable regional variations. Case-control studies have suggested that colonisation with *H. pylori* provides protection against adenocarcinoma of the oesophagus. These regional variations led us to investigate whether a relationship exists between the prevalence of *H. pylori* colonisation and the incidence rates of adenocarcinoma of the oesophagus within The Netherlands.

The prevalence of *H. pylori* was tested serologically in 1600 blood donors, 400 from each of four regions, per region divided into 5 equal ten-year age groups. The regions differed with respect to adenocarcinoma of the oesophagus incidence rates; two with low incidence rates (3.4 and 3.7/10^5 in males, 0.6 and 0.9/10^5 in females), one with intermediate (4.9/10^5 in males and 1.1/10^5 in females), and one with high (6.1/10^5 in males and 1.4/10^5 in females) adenocarcinoma of the oesophagus incidence rates.

There were clear age specific gradients in the prevalence of *H. pylori* colonisation; however, there were no differences in colonisation rates between the four regions.

This population-based study showed no relation between the prevalence rates of *H. pylori* and the incidence rates of adenocarcinoma of the oesophagus. This observation calls into question the presumed protective effect of *H. pylori* colonisation against the incidence of adenocarcinoma of the oesophagus.

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SAMENVATTING

Hoofdstuk 1. Inleiding.

De beperking van de definitie van de Barrett oesophagus tot metaplastisch cylinder epitheel van de oesophagus dat gespecialiseerd intestinale metaplasie heeft ondergaan wordt bekritiseerd op grond van het feit dat het onwaarschijnlijk lijkt dat er twee soorten metaplastisch cylinder epitheel bestaan, de een onschuldige afwijking, de andere een voorbode van kanker. Er zijn bovendien geen klinische vervolg studies die dit concept ondersteunen. In de praktijk komen beide soorten vaak samen voor1 en een Britse vervolg studie2 heeft aangetoond dat er evenveel adenocarcinomen van de oesophagus werden gevonden bij patiënten met en zonder gespecialiseerde intestinale metaplasie bij hun eerste endoscopie.

De historische sectie is vooral interessant voor mensen met gevoel voor geschiedenis. De sectie over de geschiedenis van de experimentele reflux oesophagitis beschrijft echter onderzoek na de invloed van zuur, galzuren en pancreas enzymen voor het slijmvlies van de oesophagus die momenteel nog steeds relevant zijn. Bovendien is het de moeite waard om de pogingen van de pioniers om de motiliteitstoornissen te analyseren die te grondslag liggen aan het ontstaan van reflux oesophagitis, en uiteindelijk de Barrett oesophagus, vast te leggen. Helaas heeft het succes van de proton-pomp remmers in het behandelen van de reflux oesophagitis de belangstelling voor de pathofysiologie van deze aandoening sterk doen verminderen. Hoewel technieken zoals de 24-uurs mobiele oesophageale metingen van pH, bilirubine en motiliteit en de oesophageale impedantie metingen van gas en niet-zure vloeistoffen onze inzichten in het wanneer en waar van de duodeno-gastrische-oesophageale reflux hebben vergroot, is er nog steeds weinig bekend over wat. Bedoeld wordt welke galzuren en enzymen er in welke concentraties waar aanwezig zijn op ieder gegeven ogenblik van de dag. De relevantie van dergelijke gegevens wordt aangetoond door de zeer aanzienlijke overlap tussen de mate van reflux, zoals gemeten door pH registratie en de ernst van de reflux oesophagitis of de aanwezigheid van een Barrett oesophagus. Hoewel het zeer wel denkbaar is dat er genetische factoren in het spel zijn is het toch waarschijnlijk dat de samenstelling van de refluxaat een belangrijke rol speelt bij de aanzet tot de ontstaan van zowel ontsteking als kanker.

Dit gebrek aan belangstelling voor pathofysiologie staat in schril tegenstelling tot de indrukwekkende ontwikkelingen op het gebied van de moleculaire biologie. Het inzicht in de stadia die uiteindelijk uitmonden in maligniteit en de resultaten van genetische profileringen, waarvan enkele voorbeelden besproken worden, zouden ons uiteindelijk kunnen brengen tot de Heilige Graal, het vermogen om met succes het op handen zijnde ontstaan van een adenocarcinomen van de oesophagus te kunnen voorspellen.

Hoofdstuk 2.

In dit onderzoek werden de gegevens gecombineerd van twee eerder gepubliceerde studies die door Dr Clarisse Böhmer waren verricht naar het voorkomen van gastro-oesophageale reflux ziekte door middel van 24-uur ambulante pH meting in een representatieve steekproef van willekeurig geselecteerde bewoners van tehuizen voor zeer ernstig geestelijk gehandicapte individuen, verder aangeduid als IDI. Zoals de onderzoekster had verwacht vond zij, om alsnog onbekende redenen, een zeer hoge prevalentie van zowel gastro-oesophageale reflux ziekte als van reflux oesophagitis en van Barrett oesophagus bij de IDI3. In haar tweede onderzoek had zij het aantal gevallen van oesophagus carcinoom, en specifiek dat van adenocarcinomen van de oesophagus onderzocht in deze tehuizen gedurende een periode van zes jaar4. Het huidige onderzoek had door het extrapoleren van de in de representatieve
steekproef waargenomen prevalentie van Barrett oesophagus naar de totale IDI populatie een schatting gemaakt van de omvang van de gehele IDI populatie in alle instellingen. De combinatie van deze gegevens met het aantal waargenomen adenocarcinomen van de oesophagus leverde de incidentie rate van adenocarcinomen van de oesophagus in deze populatie met Barrett oesophagus op. Deze incidentie bleek, na correctie voor leeftijdsverdeling, overeen te komen met de incidentie rate in een groep in de normale samenleving verkerende patiënten met Barrett oesophagus die al jarenlang vervolgd werden in Rotterdam. Het verschil tussen de twee groepen was echter dat de IDI niet roken en geen alcohol gebruiken. Opvallende aspecten van dit onderzoek waren dat hier voor het eerst het voorkomen van gastro-oesophageale reflux ziekte, reflux oesophagitis en Barrett oesophagus waren onderzocht bij willekeurig geselecteerde personen zonder specifieke klachten. Ook viel op dat er in vergelijking met het aantal adenocarcinomen van de oesophagus relatief weinig adenocarcinomen van de cardia waren gevonden, dit zou kunnen duiden op het feit dat roken wellicht een grotere rol speelt bij de laatst genoemde tumor.

**Hoofdstuk 3.**
Dit onderzoek naar het patroon van het voorkomen van de Barrett oesophagus wat betreft leeftijd en geslacht maakte weer gebruik van reeds gepubliceerde gegevens over het aantal waargenomen patiënten in een endoscopie afdeling die vooral onderzoekingen verrichtte voor huisartsen zodat het een zo min mogelijk op bepaalde klachten geselecteerde groep betrof. Door nu het aantal patiënten met Barrett oesophagus per 100 eerste scopieën te berekenen kon nu het patroon ontrafeld worden. Het bleek dat met de stijging van de leeftijd per jaar het voorkomen van Barrett oesophagus met een vast percentage toenam, met dien verstande dat deze stijging bij vrouwen 20 jaar later inzette dan bij mannen. Hierdoor werd ook de 2:1 verhouding tussen het voorkomen van Barrett oesophagus bij mannen en vrouwen verklaard, waarom dit verschil optrad blijft echter nog onverklaard, een hormonale invloed lijkt aanemelijk. Deze uitkomst werd onlangs door de Rotterdamse groep aan de hand van gegevens uit Nederlandse huisartsenpraktijken bevestigd.

**Hoofdstuk 4.**
Aan de hand van reeds gepubliceerde gegevens over het voorkomen van adenocarcinomen van de oesophagus in Denemarken kon door toevoeging van bevolkingsgegevens de incidentie rates van de drie soorten carcinoom van de oesophagus worden bepaald. Het betrof het adenocarcinomen van de oesophagus het adenocarcinomen van de cardia en het plaveiselcel carcinoom van de oesophagus. Hierbij bleek dat de incidentie rate van de adenocarcinomen van de oesophagus in de bevolking boven 80 plotseling daalde, uit het feit dat deze daling niet optrad bij patiënten boven de 80 met het plaveiselcel carcinoom van de oesophagus kon de conclusie worden getrokken dat er geen sprake was van een verminderde diagnostiek bij deze hoogbejaarden. De verklaring werd gezocht in een kleiner aantal personen met Barrett oesophagus in de oudere leeftijdsgroepen wat zou kunnen duiden op een geleidelijke toename van het aantal mensen met Barrett oesophagus over de afgelopen 50 jaar, een zogenaamd geboorte cohort fenomeen.

**Hoofdstuk 5.**
In aansluiting op het onderzoek beschreven in het vorige hoofdstuk werd nu met behulp van een mathematische techniek per 10 jaar leeftijd de omvang van de Deense bevolking geconstrueerd waaruit de patiënten met adenocarcinomen van de oesophagus beschreven in hoofdstuk 4 afkomstig waren. Hiermee werd een aanzet gegeven tot berekeningen van de
gehele populatie met Barrett oesophagus die momenteel slechts voor minder dan 10% bekend is. Bovendien kon nu de verklaring worden gevonden voor de daling van het voorkomen van adenocarcinomen van de oesophagus in de leeftijdsgroep boven de 80 jaar. Dit bleek inderdaad het cohort fenomeen te zijn. De door dit fenomeen voorspelde verder ontwikkeling van het aantal adenocarcinomen van de oesophagus bleek in de volgende 7 jaren uit te komen en dit betekent dat het aantal adenocarcinomen van de oesophagus jaarlijks blijft stijgen, iets dat ook al voorspeld werd door de in hoofdstuk 3 aangegeven stijging in het aantal gevallen van Barrett oesophagus.

Hoofdstuk 6.
Deze keer werd gebruik gemaakt van oorspronkelijke gegevens van de Nederlandse kankerregistratie over het voorkomen van het adenocarcinoom van de oesophagus en het adenocarcinoom van de cardia tussen 1989 en 2003. De groei in het aantal adenocarcinomen van de oesophagus en oesophagus-maag overgang bleek geheel bij zijn veroorzaakt door een sterke jaarlijkse stijging van het aantal adenocarcinomen van de oesophagus, het aantal adenocarcinomen van de cardia liep zelfs geleidelijk terug. Door een statistische bewerking kon aangetoond worden dat er geen sprake was van een verschuiving van diagnose adenocarcinoom van de cardia naar die van het mogelijk meer populair geworden adenocarcinoom van de oesophagus. Een nog niet beantwoorde vraag is of de 4:1 man-vrouw verhouding bij beide tumoren verklaard kon worden door het 20 jaar uitstel in het voorkomen van Barrett oesophagus bij vrouwen.

Hoofdstuk 7.
Uit de gegevens over het voorkomen van het adenocarcinoom van de oesophagus in Nederland, vermeld in het vorige hoofdstuk, bleek dat de incidentie rates van deze tumor sterke verschillen vertoonden tussen diverse delen van het land. Met name kwam het adenocarcinoom van de oesophagus relatief weinig voor in de oorspronkelijk Rooms Katholieke gebieden in het zuid-oosten en juist veel in de oorspronkelijk meer Protestante gebieden Rotterdam, Zeeland, Groningen, Friesland en Drente. Teneinde een meer wetenschappelijke verklaring voor deze verschillen te vinden werd de theorie getoetst dat een infectie met de Helicobacter pylori, de bacterie die verantwoordelijk is voor het ontstaan van maag- en twaalfvingerigedarm- zweren, zou beschermen tegen het ontstaan van het adenocarcinoom van de oesophagus. Hiervoor werd het voorkomen van deze infectie onderzocht bij bloeddonors uit vier verschillende gebieden waarin meer of minder adenocarcinomen van de oesophagus waren gevonden. Er bleken echter geen verschillen te bestaan zodat er sterk getwijfeld moet worden aan deze veronderstelde beschermende werking van de Helicobacter pylori.

Hoofdstuk 8.
Dit hoofdstuk omvat onder anderen deze samenvatting en conclusies. De belangrijkste conclusie is dat door de gestage toename van het aantal mensen met een Barrett oesophagus ook het aantal adenocarcinomen van de oesophagus zal toenemen waarbij wel overwogen moet worden dat slechts een klein percentage van deze mensen aan deze tumor zullen overlijden. Onderzoek naar de factoren die blijkens dit proefschrift invloed kunnen hebben op het al dan niet ontstaan van Barrett oesophagus en het daaruit voortkomende adenocarcinomen van de oesophagus lijkt aangewezen. De huidige opsporingsmethoden van mensen met Barrett oesophagus door middel van endoscopieën is waarschijnlijk ongeschikt om de grote onbekende groep van mensen met Barrett oesophagus op te sporen en hiervoor zal gezocht moeten worden naar bloedtests waarmee de veranderde expressie van genen die actief zijn in de Barrett oesophagus kunnen worden opgespoord.
Dankwoord.

De normale promovendus is uiteraard vooral dank verschuldigd aan zijn of haar medewerkers die zijn associële levenshouding aanvaarden, hem bijstaan in zijn wanhoop en hebben leren leven met zijn humeurigheid. In het geval van een promotie van achter de geraniums is er echter sprake van een heel andere medewerker en wel mijn vrouw Ursula die al deze ongemakken niet alleen in kantooruren maar 24 uur per dag heeft moeten dragen. Aan haar dus in de eerste plaats veel dank.

Het is goed te zien hoe de oude “GE centrale” zich heeft ontwikkeld tot een van de top, zo niet dé top MDL afdeling in Nederland. Dit is het werk geweest van mijn promotor, Professor Dr. Ernst Kuipers.

Beste Ernst, ik ben jou dank verschuldigd dat jij mij ondanks mijn wijsneuzerigheid toch hebt toegelaten op jouw afdeling en je bovendien hebt ingezet om deze onconventionele promotie mogelijk te maken. Daarbij hebben jouw aanmoedigingen en geloof in de goede afloop een grote rol gespeeld. Bovendien heeft jouw enorme ervaring als reviewer mij al voor menig medisch-literaire misstap behoed. Voor dit alles mijn dank.

Deze geranium reeks was nooit op gang gekomen zonder de deskundige statistische hulp van Dr. Wim Hop die zich indertijd ook belangeloos heeft gebogen over de andere artikelen van Clarisse Böhmer die de basis vormen van hoofdstuk 2. Bovendien wees jij mij de weg naar collega statisticus Ir. Caspar Looman. Voor dit alles mijn dank.

Caspar, zonder jouw bijzondere kennis en vaardigheden zouden niet alleen dit proefschrift, maar een kast vol proefschriften nooit zijn ontstaan. Het is niet overdreven te stellen dat ik jou de getallen aandraag, dat jij er vervolgens wetenschap uit distilleert en ik vervolgens als ghost writer het verhaal opschrijft, want schrijven is het enige waar jij geen zin in hebt. Caspar, het is onmogelijk jou aflopende te bedanken voor wat je hebt gedaan, niet alleen voor dit boekje maar ook voor mijn wetenschappelijke ontwikkeling.

Een belangrijke prikkel voor het wekelijkse bezoek aan de afdeling is de Barrett club van Dr. Peter Siersema. Peter, wij kennen elkaar al heel lang en hoewel de rollen nu zijn omgekeerd blijven onze literaire relaties intact. Ik ben erg trots op de manier waarop jij gedurende het interregnum de gastroenterologische wetenschap draaiend wist te houden en erg blij dat het mogelijk is gebleken jouw aandacht van de porphyrie naar de Barrett oesophagus te verplaatsen. Als co-promotor heb jij jouw rol als bad cop zeer beschaafd ingevoel. Voor dit alles dank.

Dankzij de uitvinding van de PC heeft de leescommissie het niet makkelijk gehad. In de eerste plaats wil ik Professor Dr. Guido Tytgat danken. Guido, jouw aanwezigheid als gastroenterologische wereldster op mijn promotie is alleen al een hoogtepunt. Nadat je de Nederlandse gastroenterologie op de kaart hebt gezet hebt jij mij, om nog steeds onbegrijpelijke redenen, voorzitter van de door jou tot wasdom gekomen Nederlandse Vereniging voor Gastroenterologie benoemd en daarmee een van de aangenaamste perioden van mijn leven ingeluid. Mijn dank voor jouw huidige en vroegere inspanningen.

Professor Dr. Huug Tilanus, beste Huug, jij bent de huidige vertegenwoordiger van de reeks van Rotterdamse hoogleraren in de chirurgie die ik als kleine ondernemer binnen het academisch ziekenhuis altijd tot mijn beschermheren heb mogen rekenen. Ik heb altijd diepe bewondering gehad voor jouw fantastische chirurgische vaardigheden gekoppeld aan een zeer onchirurgische bescheidenheid. Jouw magistrale boek over de Barrett oesophagus was voor mij steeds een vruchtbare bron voor op z’n minst geestelijk plagiaat. Ook hiervoor mijn dank Professor Dr. Jan Willem Coebergh, beste Jan Willem, zelden zal een zeer vrome eend zo goed in een bijt ontvangen zijn. Jouw inspirerende stroom van totaal anders gerichte conclusies en denkrichtingen heeft mij geleerd dat de epidemiology nog veel ingewikkelder is dan ik al vreesde en dat ik nog veel moet bijleren voordat ik de door jou in het vooruitzicht
gestelde titel van Epidemioloog posthuum zal kunnen aanvaarden. Mijn dank voor je inzet en wijze lessen.

Zelfs een amateur epidemioloog heeft een laboratorium nodig, dankzij de deskundige inzet van Dr. Hanneke van Vuuren en de ijver van Martine Ouwendijk konden de soms slordig en onduidelijk benoemde plasma monsters uit diverse bloedbanken toch tot ordentelijke lijst Helicobacter pylori serologie uitslagen worden omgetoverd.

Tenslotte de Barrett club. Deze groep jonge enthousiaste onderzoekers, post-docs, AIOs en studenten werd opeens met en opa-figuur geconfronteerd die bovendien iets met Peter had. Alle reden voor wantrouwen, maar integendeel, ik mag mij nu hopelijk als lid beschouwen van deze elite groep. Zo ja, mijn hartelijke dank.
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