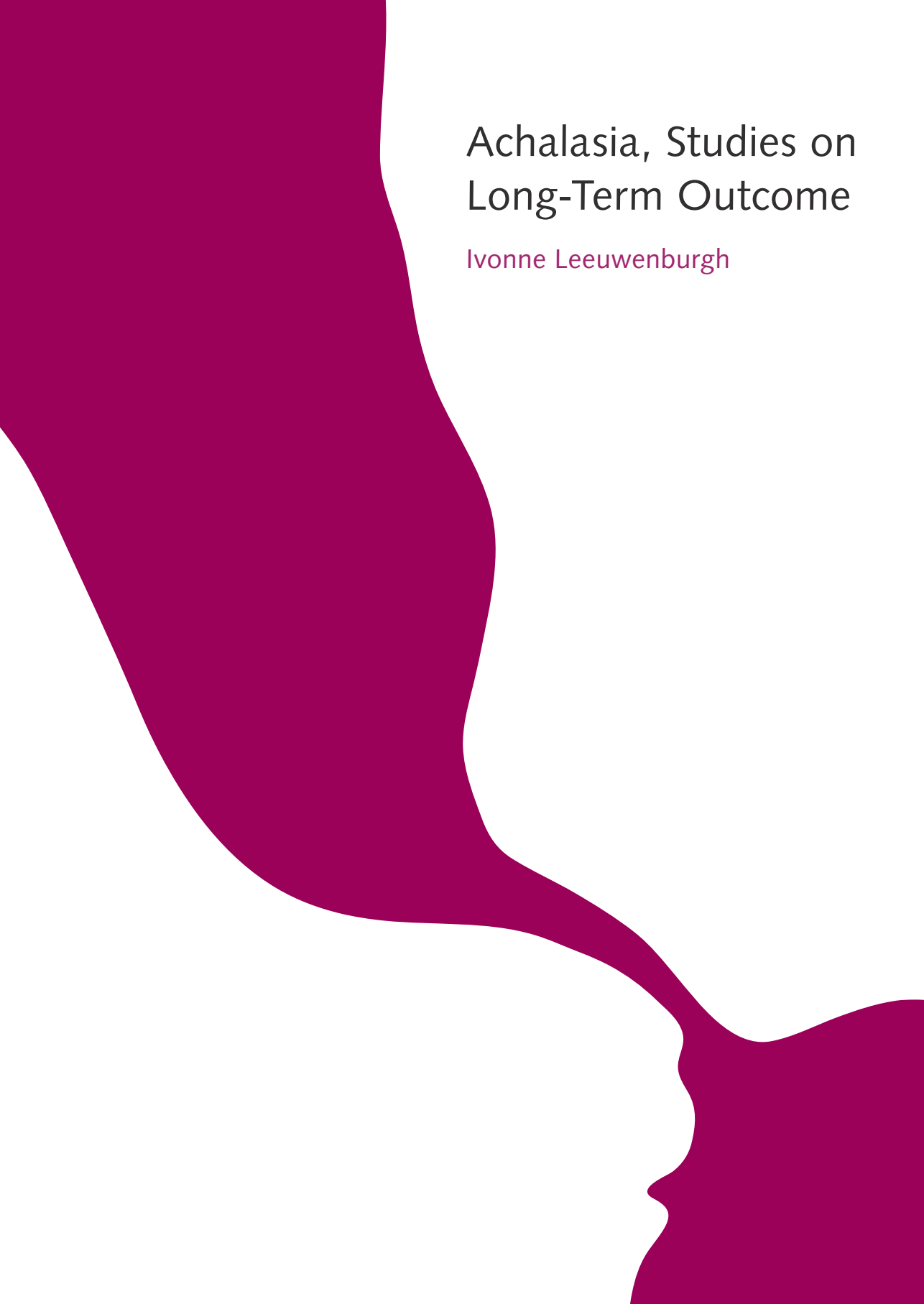
Achalasia, Studies on Long-Term Outcome

Ivonne Leeuwenburgh



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Achalasie, studies naar de lange termijn uitkomsten

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CONTENTS

Chapter 1

Introduction and aims

Achalasia is a rare condition with an annual incidence of 1 per 100.000 in the western world, an equal prevalence in men and women and a peak incidence around 60 years of age. Achalasia is a motility disorder in which failed relaxation of the lower esophageal sphincter in combination with diminished peristalsis of the distal esophagus causes a functional obstruction of the esophagus[1]. The characteristic symptoms are dysphagia for solids and liquids, regurgitation of undigested food or saliva, respiratory complaints (nocturnal cough, aspiration), retrosternal pain and weight loss. The diagnosis is made by esophageal manometry and a timed barium esophagram. Upper gastrointestinal endoscopy is performed to exclude mechanical obstruction such as due to an esophageal malignancy.

Since 1975 all patients referred to our centre with the suspicion of achalasia were analyzed, treated and followed according to a fixed protocol. More than 400 patients were included in the cohort up till now. They were diagnosed and treated by a limited number of physicians. The size and follow up of this cohort is unique and offers opportunities for research.

The etiology of achalasia is still unclear, although there are some studies suggesting an underlying autoimmune process[2, 3]. In resection specimen of patients with achalasia, inflammation was observed around the myenteric plexus[4]. Nitric oxide is implicated in the inhibitory response of the lower esophageal sphincter, however neurons containing nitric oxide are absent in the myenteric plexus of achalasia patients[5]. The inflammatory infiltrate within the myenteric plexus predominantly contains CD3-positive T-lymfocytes[4, 6], which has led to the hypothesis that an autoimmune process may underlie achalasia, leading to an inflammatory process and neuronal cell loss. HSV-1 viral antigen has been proposed as evoking agent but due to conflicting evidence the exact etiology remains undissolved[7-9]. In case of an auto-immune etiology, it is likely that other auto-immune diseases are more common in patients with achalasia. We therefore studied the prevalence of auto-immune thyroid disease in our cohort of achalasia patients and compared this to the prevalence in the general population **(chapter 8).**

Since the etiology of achalasia is still unclear, treatment is purely symptomatic aiming at improvement of food passage by lowering of the LES-pressure. There are several treatment options of which pneumatic dilatation and laparoscopic myotomy are the two most commonly applied[10]. Pneumatic dilatation is an effective treatment with low complication risk, which can be performed as an out patient procedure[11-13]. A recent randomized study with two years follow-up showed that laparoscopic myotomy and pneumatic dilatation were equally effective[14]. In **chapter 7** we report the long term success of pneumatic dilatation using our stepwise pneumatic dilatation protocol with incremental diameter balloons to 40 mm on 3 consecutive days. The aim of this study was to define predictors of success and failure to facilitate treatment choices.

Adequate treatment with sufficient symptom control does not prevent that patients suffer from persistent esophageal distension with retention of foods and fluids, bacterial overgrowth and impaired clearance of regurgitated acid gastric contents. These factors can lead

to chronic inflammation of the esophageal mucosa, which potentially increases the risk of development of hyperplasia, dysplasia and esophageal cancer [15, 16]. On the other hand lowering of LES-pressure can aggravate acid gastro esophageal reflux leading to reflux esophagitis, Barrett's metaplasia [17] and adenocarcinoma [18-20].

This suggests that inflammation is likely to be a common condition in achalasia. In **chapter 3** the incidence and severity of histological and endoscopical inflammation during follow up of achalasia patients was studied. We also investigated the association between inflammation and food stasis.

Although it is generally accepted that achalasia is a risk factor for esophageal cancer development, the reported relative risks varies between nil and 140 [21-30]. These large variations are due to differences in study design, cohort size and length of follow-up. In **Chapter 4** we report the incidence of esophageal carcinoma in our cohort and evaluate the efficacy of long term endoscopical surveillance.

As mentioned, adequate treatment of achalasia induces gastro-esophageal reflux, which can lead to Barrett's metaplasia and esophageal adenocarcinoma. Only few data exist on the development of Barrett's esophagus in patients with achalasia treated with pneumatic dilatation. In **Chapter 5** the incidence of, and risk factors for Barrett's esophagus and the development of adenocarcinoma in achalasia patients after dilatation treatment in our large cohort are determined.

Without surveillance, achalasia patients with esophageal carcinoma usually present in an advanced stage with poor prognosis. This is enhanced by the fact that achalasia patients are used to delayed esophageal emptying and only report worsening of these symptoms due to development of an obstructive tumor at a late stage [31]. Little data exist concerning the benefit of surveillance, but so far, this does not seem cost effective nor to improve prognosis [26, 28, 31, 32].

Optimization of surveillance is necessary to detect neoplastic progression at an early and curable stage. Surveillance is often difficult to perform due to several reasons. Food stasis and mucosal adherence of food makes a careful inspection difficult. Furthermore most carcinomas develop in het middle and distal third of the esophagus and therefore the whole length of the esophagus should be carefully inspected and sampled. At last, histological evaluation of dysplasia can be difficult due to the persistent presence of chronic inflammation. Probably the surveillance may be improved by the use of specific histological markers or lugol staining. In **chapter 6** we investigated whether the expression of the tumor suppressor gene p53 and proliferation marker Ki67 on surveillance biopsy samples are early predictors for malignant transformation.

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Chapter 2 General overview

Long-term risk of esophagitis, Barrett's esophagus and esophageal cancer in achalasia patients.

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ABSTRACT

Achalasia is a motility disorder of the esophagus of unknown origin, in which loss of relaxation of the lower esophageal sphincter and aperistalsis in the distal esophagus leads to a functional esophageal obstruction. The treatment is symptomatic, aiming at lowering of the LES-pressure and may be accompanied by various side effects, including gastro-esophageal reflux, a risk factor for esophagitis and its complications. Stasis and fermentation can also lead to inflammation of the esophageal mucosa, giving rise to hyperplasia of the epithelium, multifocal dysplasia and in some patients eventually squamous cell carcinoma. Unfortunately, the sensitivity and specificity of endoscopical inspection to assess inflammation or dysplasia of the esophageal lining is low, such that biopsy sampling is necessary for accurate assessment.

Although it is generally accepted that achalasia is a pre-malignant disorder, the reported increased risk of patients with achalasia to develop a squamous cell carcinoma varies from 0 – 140 times that of the normal population. In addition achalasia may predispose to Barrett's metaplasia and esophageal adenocarcinoma, which have been described in case reports after myotomy.

Surveillance-endoscopies with tissue sampling to detect pre-neoplastic lesions has been recommended, even though this can be very difficult due to mucosal adherence of food as well as hyperplastic changes of the mucosa. In the event of moderate to severe inflammation and/or persisting stasis of food despite adequate LES-pressure lowering therapy, the surveillance interval should be shortened and performed after a three-day liquid diet. The exact technique and time-intervals still need to be established, however.

KEY WORDS

Achalasia, Barrett's esophagus, carcinoma, esophagitis, reflux, surveillance,

INTRODUCTION

Achalasia is a rare condition with an annual incidence of 1 per 100,000, in which failed relaxation of the lower esophageal sphincter (LES) in combination with diminished peristalsis of the distal esophagus causes a functional obstruction of the esophagus [1]. The etiology of this disorder is still unclear, although there are some studies suggesting an underlying autoimmune process [2, 3]. The diagnosis is made by esophageal manometry and a timed barium esophagram. Upper gastrointestinal endoscopy is performed to exclude mechanical obstruction. Since the etiology is still unclear, treatment is purely symptomatic aiming at improving the passage of food by lowering of the LES-pressure. There are several treatment options of which pneumatic dilatation and laparoscopic myotomy are the two most commonly applied [4].

Despite therapy, food -stasis often persists, which may contribute to the development of chronic inflammation, dysplasia and eventually squamous cell carcinoma. For this reason achalasia is considered to be a pre-malignant disorder, however the reported risks for development of squamous cell cancer of the esophagus vary widely and the benefit of surveillance is often the subject of debate. Apart from esophageal food stasis and fermentation, esophagitis may in theory also directly result from the LES-lowering therapy, which may enhance gastro-esophageal reflux, another risk factor for esophagitis and its complications, in particular Barrett's metaplasia and adenocarcinoma.

This review focuses on esophagitis, metaplasia and dysplasia in achalasia patients, and their importance for the management and follow-up of these patients.

ESOPHAGITIS

The treatment of achalasia aims at reduction of the LES pressure to relieve dysphagia. This is usually achieved by pneumatic dilatation or myotomy, techniques which may induce significant sphincter insufficiency. This may in theory lead to significant gastro-esophageal reflux, potentially leading to microscopic and macroscopic signs of esophagitis. On the other hand insufficient treatment of the non-relaxing sphincter is associated with chronic stasis and intraluminal fermentation of food, which may also predispose to esophagitis. This intricate balance suggests that esophagitis is likely to be a common condition in achalasia patients, yet data regarding this issue are very scarce.

Reflux after surgical myotomy may occur in up to 60 % of patients, and for many surgeons this is a reason to combine myotomy with a fundoplication. Reflux after pneumatic dilatation has not been widely studied, and is reported to be less than 10 % in the few available reports [5-8].

About half of the newly diagnosed achalasia patients complain about pyrosis prior to any therapy aiming at reducing LES pressure [9]. Abnormal acid exposure before LES-lowering

therapy has been reported by Shoenut et al in 10 of 48 (21%) achalasia patients and by Smart et al in 1 of 17 patients [10, 11]. This abnormal acid exposure could be due to gastro-oesophageal reflux, but can also be caused by ingestion of acidic food or lactic acid production after bacterial fermentation of retained food [11]. Differentiation between these two mechanisms can be easily made by 24-hour intra-esophageal pH-monitoring. Patients with gastro-esophageal reflux show sharp pH drops, whereas a steady drift of the pH to below pH 4 is typical for fermentation [12, 13]. It is most likely that the reported abnormal acid exposure in achalasia patients is caused by the latter in particular [11, 12].

We previously evaluated the incidence of esophagitis in 1102 biopsy samples of 251 patients with achalasia, treated with pneumatic dilatation. Forty percent of patients developed moderate to severe esophagitis (Ismail-Beigi grade 2 or 3) after a mean follow-up of 8.4 years [14]. We showed that this inflammation was indeed caused by food stasis and that the sensibility of endoscopy to assess inflammation was quite low [15].

Our results show that inflammation is a common finding in patients with achalasia treated with pneumatic dilatation. The assumption that inflammation predisposes to hyperplasia and dysplasia underlines the necessity to obtain esophageal biopsy samples during follow-up of achalasia patients to score for esophagitis [16]. Histological signs of moderate to severe esophagitis in combination with food stasis, or moderate to marked food stasis alone, form an indication for retreatment if possible. Nowadays, most clinicians wait for the patient to report symptom worsening before offering repeated LES dilatation. However, it is well known that achalasia patients tend to underreport their symptoms for several reasons. This includes the fact that they are used to symptoms of poor esophageal clearance, which often has already existed for a very long time even before the first treatment is given. Other reasons for underreporting symptoms are the discomfort of treatment and, in particular in elderly achalasia patients, an altered vagal afferent response leading to a diminished perception of pain [17, 18].

BARRETT'S METAPLASIA AND ADENOCARCINOMA

Barrett's metaplasia and achalasia appear as contradictions, but the co-existence of these disorders has been reported in approximately 30 cases in the literature, most of them after myotomy and a few after dilatation [19, 20]. Adenocarcinoma in Barrett's metaplasia in achalasia has also been described [21, 22]. In addition, there are some case reports of achalasia patients with Barrett's metaplasia at the time of presentation even before LES-lowering therapy [20, 23]. The pathogenesis of Barrett's metaplasia in these patients is uncertain, but there are several potential mechanisms. First, intestinal metaplasia could be a complication of LES-lowering therapy inducing sphincter insufficiency and pathological reflux. Secondly, refluxed acid cannot be cleared from the a-peristaltic esophagus. Thirdly it could be the result of persistent acidic conditions due to fermentation of retained food. Finally, it is possible that the Barrett's metaplasia existed prior to the development of achalasia [24].

In our series of 331 achalasia patients treated with pneumatic dilatation 28 (8.5%) developed endoscopical evidence of Barrett's metaplasia with intestinal metaplasia in the histological samples. Patients with a hiatal herniation were more likely to develop Barrett's metaplasia (28% versus 3%). Also, a lower LES-pressure after dilatation predisposed to Barrett's metaplasia (13.9 versus 17.4 mmHg) [25]. This implies that pathological acid gastro- esophageal reflux is probably the main cause of development of Barrett's metaplasia. One patient developed high-grade dysplasia and in 3 patients an adenocarcinoma was detected during follow-up. In the total group of 331 patients with achalasia the long-term incidence of adenocarcinoma was 1% [25].

ESOPHAGEAL CARCINOMA

Achalasia is considered to be a pre-malignant disorder, although the reported risk of developing carcinoma varies enormously. Several autopsy studies have reported an esophageal neoplasia prevalence of 20 –29% in achalasia patients [26-28]. The largest cohort follow-up study was conducted by Wychulis in 1318 patients with achalasia followed for 17 patient-years. This study revealed a seven-fold increased risk for esophageal cancer compared to the general population [29]. Other follow-up studies report oesophageal cancer incidences varying from nil per [953] patient-years to [1] per [173] patient-years [30-32]. This translates into relative risks varying from zero to 140 times increased compared to the sex- and age-adjusted population. An analysis of our group in 1995 on 195 patients followed for 4.5 years showed an esophageal cancer incidence of 1 per 293 patient-years [31]. A direct comparison of all the conducted studies is difficult, because they differ in design and in their definitions of the actual start of follow-up, varying from the start of symptoms versus start of treatment.

Patients with achalasia who develop esophageal cancer often have a dismal prognosis. This is at least in part due to the fact that these patients are used to symptoms of impaired food passage and often report worsening too late [33]. This knowledge and the observation that the annual risk for cancer is comparable to the annual risk of developing neoplastic lesions in other inflammatory diseases of the gastro-intestinal mucosa, like Barrett's metaplasia, warrant the consideration for surveillance. The benefit of surveillance strategies in achalasia is often argued. Surveillance should aim at both detection of neoplastic transformation at a curable stage and identification of patients mostly at risk of developing such a lesion, e.g patients with persistent severe inflammation or food stasis despite LES-lowering therapies and those with Barrett's metaplasia. Surveillance in achalasia, however, is rather difficult to accomplish due to several reasons. First, stasis and mucosal adherence of food compromise a careful inspection. In case of severe stasis, endoscopy should be done after a three-day liquid diet, even more so because severe food stasis is probably a risk factor for cancer development. Secondly most carcinomas develop in the middle and distal third of the esophagus and therefore, in contrast to Barrett's esophagus, the whole length of the esophagus should

be sampled [34]. Thirdly the surveillance-interval and the start time of follow-up need to be established, because the risk of cancer is related to duration of symptoms.

In our opinion, surveillance endoscopy with tissue sampling should be performed in patients with longstanding achalasia, probably every three years. This surveillance should start no later than 15 years after onset of symptoms. In case of moderate to severe inflammation and/or stasis of food persisting despite adequate LES-pressure lowering therapy, the surveillance interval should be shortened and the endoscopy should be performed after a three-day liquid diet. Lugol-staining may be of use in spotting suspicious areas and allow better sampling[35].

However, the exact technique and the time-intervals for surveillance need to be determined.

CONCLUSIONS

After pneumatic dilatation, a considerable proportion of patients with achalasia develop moderate to severe esophagitis. This inflammation is caused by stasis of food and can lead to dysplasia and development of squamous cell carcinoma.

Barrett's metaplasia and adenocarcinoma can also develop in treated achalasia patients. The cause is still unclear, but pathological acid gastro-esophageal reflux is probably an important factor.

The risk of development of squamous cell carcinoma is substantially increased in achalasia patients compared to the healthy population.

Surveillance is likely to be useful in detecting neoplastic lesions at a curable stage and in identifying the patients most at risk of developing such a lesion, e.g. those with persistent food stasis or moderate to severe inflammation. The optimal surveillance-technique and timing still need further study and improvement.

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Chapter 3 Inflammation

Esophagitis is common in patients with achalasia after pneumatic dilatation

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ABSTRACT

Background: Achalasia, an esophageal motor disease, is associated with functional esophageal obstruction. Food stasis can predispose for esophagitis. Treatment aims at lowering of the lower esophageal sphincter pressure, enhancing the risk of gastro-esophageal reflux. Nevertheless, the incidence of esophagitis after achalasia treatment is unknown.

Methods: A cohort of 331 patients with achalasia were treated with pneumatic dilatation and followed. Esophagitis and stasis were assessed by endoscopy and inflammation was graded by histology.

Results: 251 patients were followed for a mean 8.4 yrs (range 1-26). The average number of endoscopies with biopsy sample sets per patient was 4 (range 1-17). Three patients had no histological signs of esophagitis throughout follow-up, 139 had esophagitis grade 1, 49 esophagitis grade 2, and 60 grade 3. Specialized intestinal metaplasia was found in 37 patients. The association between endoscopic food stasis and histological inflammation was significant. The association between endoscopic signs of esophagitis and histological inflammation was poor.

Conclusions: Forty percent of the achalasia patients develop chronic active or ulcerating esophagitis after treatment. Inflammation was associated with food stasis. Since the sensitivity of endoscopy to detect inflammation is low, surveillance endoscopy with biopsy sampling and assessment of stasis is warranted to detect early neoplastic changes.

KEYWORDS:

Achalasia, esophagitis, inflammation, pneumatic dilatation, surveillance

INTRODUCTION

Achalasia is an uncommon disorder with a largely unknown aetiology, in which there is a loss of relaxation of the lower esophageal sphincter and a-peristalsis of the distal part of the esophagus [1]. Treatment options are merely symptomatic and aim at lowering the LES -pressure to improve the passage of fluid and food. Pneumatic dilatation and laparoscopic myotomy are the two most commonly applied treatment modalities. Pneumatic dilatation is an effective treatment with low complication risk and can be performed as an outpatient procedure [2-4].

Lowering LES-pressure not only intends to improve food passage but may also increase gastro-esophageal reflux. Such reflux is thought to be quite common after surgical myotomy, for this reason myotomy is often combined with an anti-reflux procedure [5]. A previous study has reported that symptomatic reflux also may occur in patients after pneumatic dilatation [6]. Complications of gastro-esophageal reflux, such as Barrett's metaplasia and adenocarcinoma of the esophagus in patients treated for achalasia are incidentally reported [7-9].

Patients with achalasia also have an increased risk of developing squamous-cell carcinoma of the esophagus. This is probably due to chronic inflammation and hyperplasia of the epithelium in response to stasis of food and fluid [10]. In esophageal resection-specimens from patients with achalasia and esophageal squamous-cell carcinoma, a marked hyperplasia was found together with multiple dysplastic foci, which findings supports this hypothesis [11].

Even though achalasia patients thus have various significant risk factors for the development of chronic esophagitis, a condition with long-term implications, little is known about the prevalence of this disorder in achalasia patients during follow-up. We therefore studied the incidence and severity of esophagitis in patients treated with pneumatic dilatation.

MATERIAL AND METHODS

Patients

Between 1975 and 2003 all patients with achalasia referred to the Erasmus MC Rotterdam were diagnosed, treated and followed according to a strict protocol which did not change over time and which was carried out by a limited number of physicians. Achalasia was diagnosed by esophageal manometry. Aperistalsis of the distal part of the esophagus and loss of relaxation of the lower esophageal sphincter were considered diagnostic for achalasia. This diagnosis was supported by a timed barium esophagram in which stasis of contrast, a bird beak appearance and widening and elongation of the esophagus were considered compatible with achalasia. An upper gastro-intestinal (GI) endoscopy was performed to rule out secondary achalasia resulting from a neoplastic lesion. The treatment protocol consisted of three dilatation sessions on three consecutive days using Rigiflex balloon dilator (Boston Microvasive) with an increasing diameter of 30, 35 and 40 mm. The balloon was positioned in

the LES under fluoroscopic control and easily insufflated to a pressure of 300 mm Hg and kept insufflated for 1 minute. During insufflation there was attention for a waist and its possible disappearance. Treatment success was defined as relieve of symptoms (in particular regurgitation, vomiting and weight loss) regardless of improvement of the esophagram. Disease recurrence, was defined as deterioration of symptoms, and was primarily treated with pneumatic dilatation**,** whereas a myotomy was performed in patients who had responded poorly to previous dilatation. Patients were seen after 1, 2, 4 and 7 years of follow-up. This included recording of the medical history, use of medication and body weight. In addition esophageal manometry, upper G-I endoscopy and a timed barium esophagram was performed. Seven years after initial treatment, follow-up was continued three-yearly by upper G-I endoscopy with biopsy sampling.

Upper G-I endoscopy and biopsy sampling

At each upper G-I endoscopy, the esophagus and gastro-esophageal junction were inspected for the presence of macroscopic esophagitis and, in a proportion of the endoscopies posterior, graded according the Los Angeles classification (grade A-D)[12], Barrett's metaplasia, carcinoma, and stasis of food (mild in case of retention of fluid only, moderate in case of some fluid and solids and severe when there is massive retention in which it was impossible to inspect the mucosa). Three to four biopsy specimens were sampled with standard forceps just above the gastro-esophageal junction. If there was suspicion of columnar metaplasia, dysplasia or carcinoma, additional biopsies were obtained**.** In case of severe stasis, extensive irrigation and suction were applied to clean the esophagus. When this remained unsuccessful and proper examination of the mucosa remained impossible, the endoscopy was repeated after three days of liquid diet.

Histology

About 4 µm hematoxylin-eosin stained routine histological sections were used. An experienced G-I pathologist blinded to the clinical data re-evaluated all samples. Esophagitis was assessed according to established criteria[13-15]. Using these criteria, the following lesions were considered compatible with reflux disease of increasing severity: (1) basal layer hyperplasia (2) elongation of papillae, (3) dilation of papillary vascular spaces, (4) intraepithelial inflammatory infiltration, (5) mucosal erosion and (6) granulation tissue. For practical purposes inflammation was graded into chronic (i.e. esophagitis grade 1, in the presence of criteria 1-3), chronic active (i.e. esophagitis grade 2, in the presence of criteria 4 with or without criteria 1-3), or eroding ulcerating (i.e. esophagitis grade 3, in the presence of criteria 5 or 6)(Figure 1) In addition to this inflammatory score, the presence of intestinal metaplasia, dysplasia or neoplasia was recorded.

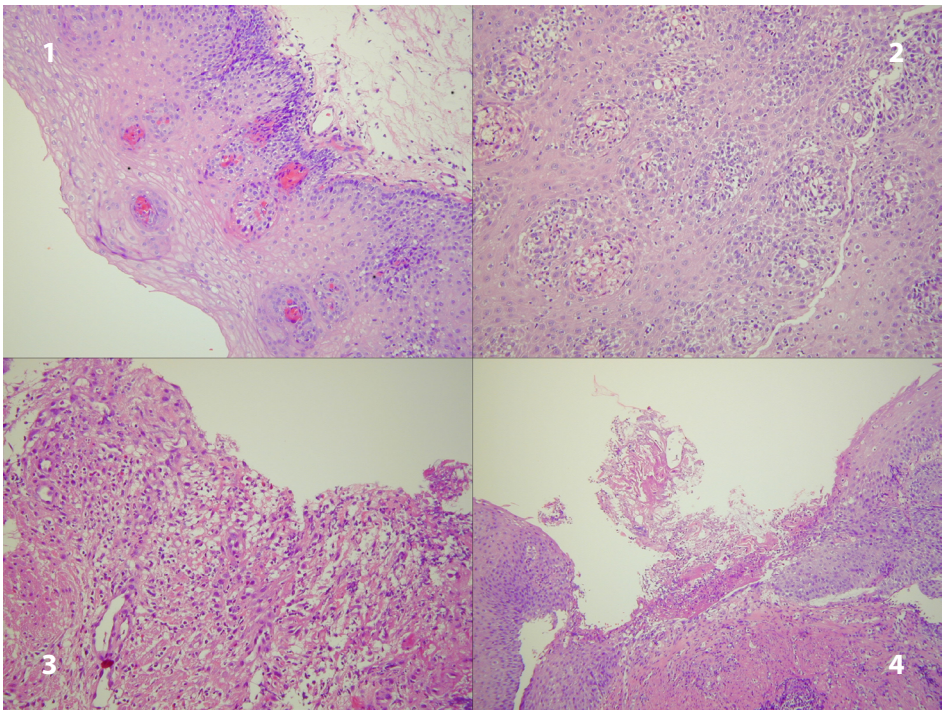

Figure 1: Esophageal biopsy samples, H-E-stained; all magnifications 20 x objective except (4): 10 x objective. (1) Epithelial hyperplasia, elongation of papillae and dilated vascular spaces (esophagitis grade 1). (2) Intraepithelial inflammatory infiltrate with an occasional eosinophil (esophagitis grade 2). (3) Granulation tissue (esophagitis grade 3). (4) Erosion and ulceration (esophagitis grade 3)

Statistics

In order to assess the association between histological inflammation and stasis, we applied a logistic regression model with random effect to correct for the fact that in each patient a different number of biopsy samples were taken. We assumed an autoregressive correlation structure between measurements within a patient applying Proc Genmod with the repeated statement in SAS 8.2. Correction for age, which had a significant effect, was made. The effect of smoking behaviour was not significant and therefore no correction was made in a group with a surprisingly low number of smoking patients. To assess the association between microscopic and macroscopic inflammation we used this logistic regression model in a similar way. P-values < 0.05 were considered significant.

RESULTS

Between 1975 and 2003, 331 patients (160 male, mean age 50.3 yrs, range 4-92) with achalasia were treated with pneumatic dilatation. Seventy-two patients (22 %) were excluded from this analysis for reasons of missing data (n= 37), or because biopsy sampling was not performed (n= 35). In total, 259 patients were included in the final analysis. A total of 1238 biopsy sample

sets (containing 4333 biopsy specimens) had been obtained from these patients, 111 sample sets were excluded from the analysis because the slides could not be found in the archive (n=25), the slides were technically not evaluable (n=3), in the slides no squamous epithelium or intestinal metaplasia or carcinoma was present (n=59), or a missing endoscopic report (n=24). In 25 sample sets, no squamous epithelium was present and therefore inflammation could not be assessed but there were signs of intestinal metaplasia or carcinoma. In total, in 1102 samples from 251 patients sufficient squamous epithelium allowed histological assessment of inflammation and the corresponding endoscopic report was available. The mean follow-up of these patients was 8.4 years (range 1 - 26). A mean of 4 sample sets per patient had been obtained over time (range 1 - 17).

One hundred-sixty-five (65,7 %) patients had no endoscopic signs of esophagitis throughout the observation period. 55 (21,9 %) showed endoscopical esophagitis grade A, 17 (6,8 %) grade B, 9 (3,6 %) grade C and 5 (2 %) showed grade D. Twenty-five (10 %) patients developed endoscopical signs of a segment of columnar epithelium in the distal esophagus suggesting Barrett's metaplasia. In 13 (5%) patients, a solitary ulcer was seen at least at one occasion during follow-up.

Histological signs of esophagitis were scored according to the highest level of inflammation per patient during follow-up. Chronic active or ulcerating esophagitis (grade 2 (n=49) or 3 (n=60)) was found in 109/251 (43.4%) patients during follow-up. Chronic esophagitis (grade 1) was seen in 139 (55.4%) patients and only 3 (1.2%) patients had no inflammation (grade 0) during follow-up. After a mean of 12 years of follow-up (range 7 – 17) fifty percent of the patients developed moderate to severe inflammation (being grade 2 or 3) in the biopsy samples. (Figure 2)

Low-grade dysplasia (LGD) in squamous cell epithelium developed in 11/251 (4.4%) patients after a mean follow-up of 6 years (range 0-12) after pneumatic dilatation and 14.5 years (range 9-29) after the start of symptoms. One of these patients developed squamous cell

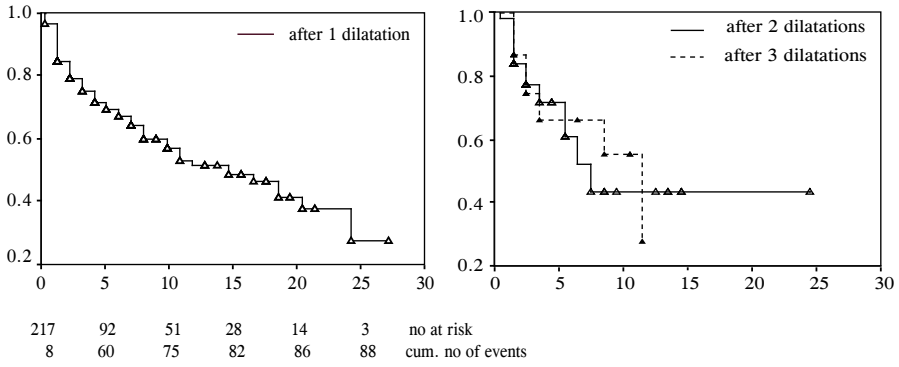

Figure 2: probability of survival without moderate to severe esophagitis as assessed by histology in years after the first dilatation series (1) and after first (2) and second (3) retreatment because of symptom recurrence.

carcinoma 33 year after the start of symptoms, whereas 1 patient developed carcinoma in situ 29 years after the start of symptoms. Squamous cell carcinoma developed in 7/251 (2.8%) patients after a mean follow-up of 20.4 years (range 2-33) after the start of symptoms and 10 years (range 1-23) after the initial treatment.

Barrett's metaplasia (defined as the presence of intestinal metaplasia) was histological detected in 37/251 (14.7%) patients after a mean follow up of 6.0 years (range 0-28) after initial treatment. From these 37 patients, 12 (32.4%) developed LGD, 1 high-grade dysplasia and 3 patients adenocarcinoma.

The estimated predicted value for the presence of histological inflammation in the presence of endoscopic esophagitis was only significant for grade B-D (p = 0.0013). In 28/53 (52.8%) of the samples, taken from an esophagus with endoscopic esophagitis grade B, C or D, histological inflammation grade 2 or 3 was found. However, 156/895 (17.4%) of the biopsy samples obtained from patients without endoscopic esophagitis also showed grade 2 or 3 inflammation. (Table 1, Figure 3)

	Inflammation		
Esophagitis	**Odds ratio**	**95% Confidence interval**	**p-value**
None	1.0		
Grade A	1.43	0.86 – 2.38	0.17
Grade B t/m D	3.92	1.71 – 9.01	0.0013

Table 1: Prediction of histological inflammation with upper and lower confidence limit in the absence of endoscopical signs of esophagitis (0) and in the presence of esophagitis grade A (1) and esophagitis grade B, C and D (2)

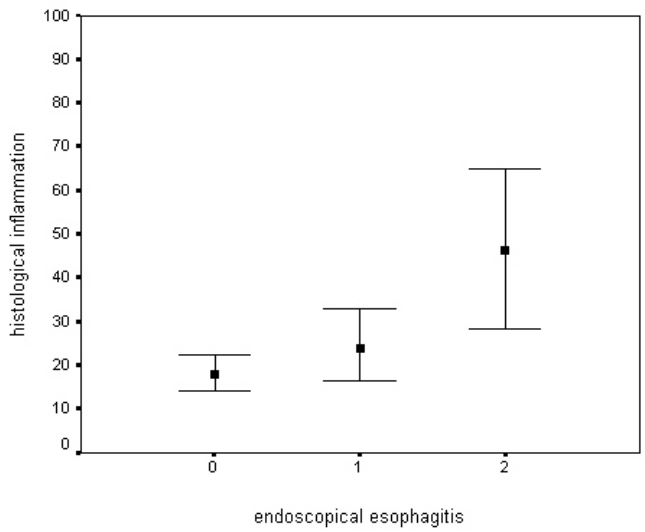

Figure 3: Prediction of moderate to severe (grades 2 and 3) histological inflammation (%) with upper and lower confidence limit in the absence of endoscopical signs of esophagitis (0) and in the presence of esophagitis grade A (1) and esophagitis grade B, C and D (2)

Assuming the histological investigation as the gold standard for the assessment of inflammation, the sensibility and specificity of endoscopy for esophagitis was 37.6 % (range 31 – 44) and 83.6% (range 81 – 86) respectively.

The odds ratios showed a significant association between food stasis and the grade of histological inflammation, which was also present after correction for age and number of samples per patient. With increasing severity of food stasis during endoscopy, the odds ratio increased up to 4.5 in case of severe stasis ($p<0.0001$). (Table 2, Fig 4)

In this study, which started in 1975, different acid suppressive therapies were used, usually for short periods after diagnosing esophagitis at endoscopy. In almost all cases, these therapies had been withdrawn long before the next surveillance endoscopy was performed and individual patients used different kinds of acid suppressive therapies during follow-up. We analysed the data of medication use, which was recorded in the medical chart. If there was no information regarding medication, we assumed no acid lowering medication was

	Inflammation		
Stasis	**Odds ratio**	**Confidence limits**	**P-value**
0	1.0		
1	1.57	1.00 – 2.46	0.049
2	2.58	1.45 – 4.59	0.0012
3	4.50	2.57 – 7.90	<0.0001

Table 2: Prediction of histological inflammation with upper and lower confidence limits when there is no stasis (0), mild stasis (1), moderate stasis (2) and severe stasis (3)

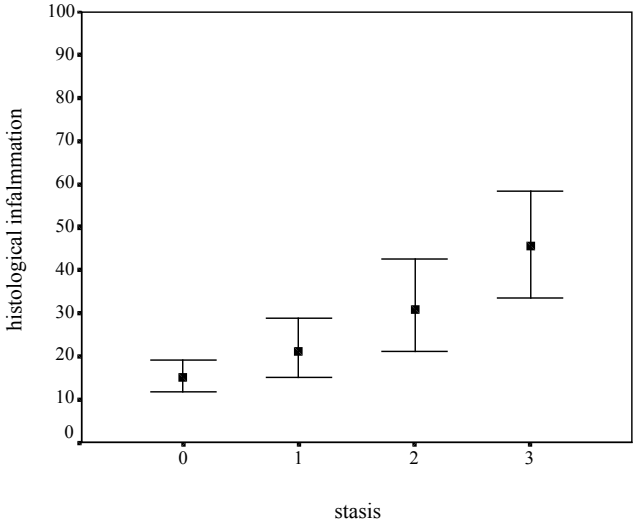

Figure 4: Prediction of moderate to severe (grades 2 and 3) histological inflammation (%) with upper and lower confidence limits in patients who either have no food stasis (0), mild stasis (1), moderate stasis (2) and severe stasis (3)

taken. We made a distinction between proton pump inhibitors (ppi), H_2- antagonist and other (mostly topical) medication.

Sixty-eight (27%) patients used acid suppressive therapy at any time during a follow-up endoscopy, thus 243 (22%) of the 1102 endoscopies were performed while the patient was using acid suppressive therapy (89 endoscopies in 32 patients during PPI treatment, 81 endoscopies in 30 patients during H2-antagonists, and 73 endoscopies in 35 patients during other treatment). Patients taking acid lowering therapy other than PPI's or H2-antagonists showed significantly more inflammation grade 2 and 3 than the untreated group (34 versus 18%, p=0.001). The difference in prevalence of inflammation grade 2 and 3 between the patients on PPI's or H2-antagonists versus the untreated patients was not significant (17% and 26% versus 18%; p=0.720, resp 0.110)

DISCUSSION

The treatment of achalasia aims at reduction of the LES-pressure to relieve dysphagia. This is usually achieved by pneumatic dilatation or myotomy, techniques which may induce significant sphincter insufficiency. Gastro-esophageal reflux, which may result from this insufficiency, is able to cause esophagitis. On the other hand insufficient treatment of the non-relaxing sphincter may lead to stasis of food, which can also predispose to esophagitis. This intricate balance suggests that esophagitis is likely to be a common condition in achalasia patients, yet there has to our knowledge no data regarding this issue been published.

Our results show that chronic active esophagitis is indeed very common in achalasia patients after dilatation therapy. In our series, nearly all patients showed histological evidence of chronic esophagitis. Most of them showed active inflammation with erosive histological lesions, and these lesions largely remained unchanged over time. The prevalence of inflammation was lower when judged by endoscopy, however also endoscopy showed macroscopic evidence of esophageal inflammation in a considerable proportion of patients. Thirty-six percent of patients showed macroscopic signs of esophagitis, being grade B to D in 12% of them. The sensitivity of endoscopy to detect inflammation of the esophageal mucosa is low, which among others, can be explained by limitations of endoscopical inspection in patients with often mucosal adherence of food, even after fasting. We applied the Los Angeles classification because of its widespread application and usefulness in clinical practice, even though this classification was not primarily developed for patients with food stasis and achalasia.

It is difficult to differentiate between inflammation caused by acid reflux or food stasis. It is well known that achalasia patients even at first presentation prior to therapy can complain of heartburn [16, 17]. The etiology of which is still unclear. The histological picture did not allow a clear differentiation and therefore we differentiated by endoscopy on the basis of the presence of food stasis on one hand versus esophagitis without evidence of food stasis on the other.

Stasis of food is common in achalasia patients, also after dilatation treatment. This condition is often neglected as irrelevant when not accompanied by deterioration of symptoms. We however observed a significant association between stasis of food and the histological presence of inflammation. Chronic stasis is thought to contribute to mucosal hyperplasia, which can be complicated by multifocal dysplasia. This may after many years give rise to an increased risk of developing a squamous-cell carcinoma [11]. Together, this warrants a more active approach towards achalasia patients with persisting moderate or marked stasis of esophageal contents. In order to reduce chronic inflammation and its complications, patients with chronic stasis or eroding ulcerative esophagitis may require repeated dilatation treatment or myotomy, probably even independent of their reported symptoms. The association between symptoms, esophagitis and stasis is only moderate. Patients with achalasia tend to underreport their symptoms, because they are used to an often long-standing situation of dysphagia and interpret a slight improvement in esophageal emptying as already a dramatic improvement [3, 18]. Besides, many achalasia patients, especially those who are older have an altered vagal afferent response leading to a diminished perception of pain [19, 20].

In order to detect inflammation, surveillance endoscopy with adequate biopsy sampling is necessary. The interval between the surveillance endoscopies needs further investigation and this may be dependent on the level of inflammation. The importance of grade 1 inflammation (basal layer hyperplasia, elongation of papillae and dilation of papillary vascular spaces) which occurred in nearly all our patients is likely to be limited. But when more severe inflammation such as histological grade 3 (granulation, ulceration) remains present despite adequate therapy, more intensive surveillance should be offered, probably every year starting 10 years after the onset of symptoms, when it is known that the risk of developing a carcinoma is increasing[21, 22]. Since surveillance endoscopies can be technically very difficult in achalasia patients, we should prescribe a three-day liquid diet before the endoscopy to allow better inspection of the esophageal mucosa. During endoscopy, the use of lugol-staining may help to identify high-risk lesions and allow directed biopsy sampling [23]. When LGD is detected during endoscopy, surveillance intervals should be intensified and in case of HGD we suggest esophagectomy because of the frequent multifocal character of the dysplasia [7].

Our study did not provide an answer to the question whether a symptomatic relapse of achalasia affects esophagitis. This limitation was due to the fact that in a great proportion of our patients no samples were taken at the time of symptom relapse and mostly these relapses occurred between surveillance intervals.

There was no significant difference in inflammation between patient using PPI's or H2 antagonists and the patients without acid lowering therapies. However we analysed the data of medication use as recorded in the medical chart. If there was no information regarding medication, we assumed no acid lowering medication was taken, which may be a wrong conclusion. Besides different kinds of acid-lowering therapies were used for usually small

periods in our patient groups and individual patients used different kinds of acid suppressive therapies during follow-up.

CONCLUSIONS

Our study shows that esophagitis is a very common condition in patients with achalasia. Almost all patients with achalasia treated with pneumatic dilatation develop chronic esophagitis during follow-up. The sensitivity of endoscopy for assessment of esophagitis in these patients is poor, but there is a significant association between endoscopic signs of food stasis and the presence of esophagitis. The latter may lead to hyperplasia, dysplasia and development of squamous cell carcinoma. For this reason, the presences of food stasis or histological esophagitis grade 3 forms in our opinion an indication for retreatment by pneumatic dilatation or myotomy even in the absence of deterioration of symptoms.

This warrants a more active approach in the follow-up of patients with achalasia and requests for surveillance endoscopy with biopsy sampling in patients with achalasia after treatment with pneumatic dilatation to evaluate stasis and inflammation in biopsy samples.

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Chapter 4 Esophageal cancer

Long term esophageal cancer risk in patients with primary achalasia; a prospective study

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ABSTRACT

Introduction: Achalasia patients are considered at increased risk for esophageal cancer, but the reported relative risks vary. Identification of this risk is relevant for patient management. We performed a prospective evaluation of the esophageal cancer risk in a large cohort of achalasia patients with long-term follow-up.

Methods: Between 1975 and 2006 all patients diagnosed with primary achalasia in our hospital were treated and followed by the same protocol. After graded pneumatic dilatation, all patients were offered a fixed surveillance-protocol including GI endoscopy with esophageal biopsy sampling.

Results: We surveyed a cohort of 448 achalasia patients (218 men, mean age 51 yrs at diagnosis, range 4 to 92 years) for a mean follow-up 9.6 years (range 0.1 – 32). Overall 15 (3.3%) patients (10 men) developed esophageal cancer (annual incidence 0.34 (95% confidence interval 0.20-0.56)). The mean age at cancer diagnosis was 71 years (range 36-90) after a mean of 11 years (range 2 – 23) following initial presentation, and a mean of 24 years (range 10 - 43) after symptom onset. The relative hazard rate of esophageal cancer was 28 (confidence interval 17 – 46) compared with an age- and sex- identical population in the same timeframe. Five patients received a potential curative treatment.

Conclusion: Although the gastro-esophageal cancer risk in patients with longstanding achalasia is much higher than in the general population, the absolute risk is rather low. Despite structured endoscopical surveillance, most neoplastic lesions remain undetected until an advanced stage. Efforts should be made to identify high risk groups and develop adequate surveillance strategies.

KEY WORDS:

esophageal achalasia, therapy, pneumatic dilation, malignancy, esophageal cancer, surveillance, Barrett's esophagus, adenocarcinoma, squamous cell cancer.

Study highlights:

1 *What is current knowledge:* Achalasia may be a risk factor for esophageal cancer development. Surveillance is debated.
2 *What is new here:*In our large cohort with long term follow-up, the relative hazard rate for development of esophageal cancer in achalasia patients was 28. Despite regular surveillance with 1- to 3- years intervals, most cancer are detected in advanced state

INTRODUCTION

Achalasia is a rare, chronic esophageal motility disorder with an estimated annual prevalence of 1 per 100,000 subjects in the western populations. The disease can occur at all ages but the incidence seems to increase with age. Predominant symptoms are dysphagia and regurgitation, due to the impaired relaxation of the lower esophageal sphincter (LES) and the loss of normal peristalsis. Treatment is purely symptomatic as the etiology of achalasia is still unclear. Treatment aims at lowering of the LES-pressure to improve the passage of food [1]. Adequate treatment with sufficient symptom control does not prevent that patients suffer from persistent esophageal distension with retention of foods and fluids, bacterial overgrowth and impaired clearance of regurgitated acid gastric contents. These factors can lead to chronic inflammation of the esophageal mucosa, which potentially increases the risk of development of hyperplasia, dysplasia and esophageal cancer [2, 3]. In contrast, lowering of LES-pressure can aggravate acid gastro esophageal reflux leading to Barrett's metaplasia[4] and adenocarcinoma [5-7].

The relationship between achalasia and esophageal carcinoma has been studied before. Three studies did not observe esophageal cancer in achalasia patients [8-10]. However, these follow-up studies contained rather low numbers of evaluable cases (91 and 162 respectively) and had a limited follow-up of 10 years or less. Another study including 253 patients found no significant difference in 20 year survival rate between achalasia patients and controls[11] In contrast, other investigators reported an increased risk for development of esophageal cancer, but the results of these studies nevertheless varied widely. Again, some of these studies were limited in follow-up [12-14] or patient numbers [15]. The largest cohort follow-up study however reported on 1318 patients with achalasia followed for 17 patient-years. This study revealed a sevenfold increased risk for esophageal cancer compared to the general population [16]. Another study conducted in Sweden included 1,062 patients followed for 9,864 patient years and found a sixteen fold increased esophageal cancer risk[17]. In addition to these cohort studies, autopsy studies reported 20-29 % of patients with achalasia having esophageal cancer at death [18-20]. Altogether, the reported relative risk for esophageal cancer varied between zero and 140. This variation may be due to differences in study design (cross sectional versus follow-up), numbers of patients and length of follow-up. Also, patients with pseudo-achalasia due to esophageal or gastric cancer were not always excluded and most studies did not mention other risk factors for developing esophageal squamous cell carcinoma such as alcohol and nicotine use.

Despite these conflicting data achalasia of the esophagus is generally accepted as a condition with an increased risk of particularly squamous cell carcinoma, which raises the question whether surveillance is needed and useful.

Without surveillance, achalasia patients with esophageal carcinoma usually present in an advanced stage with poor prognosis. This is enhanced by the fact that achalasia patients are

used to delayed esophageal emptying and only report worsening of these symptoms due to development of an obstructive tumor at a late stage [21]. Little data exist concerning the benefit of surveillance, but so far, this does not seem cost -effective nor to improve prognosis [13, 15, 21, 22]. The most recent American Society for Gastrointestinal Endoscopy guidelines do not recommend routine endoscopic surveillance in the absence of more convincingly supportive data[23].

We performed a large prospective study (with 448 patients and 4,483 patient years of follow-up) with strict follow-up to determine the incidence of esophageal carcinoma and to evaluate the efficacy of long-term endoscopical surveillance.

PATIENTS AND METHODS

Study patients

This single centre cohort study was performed in a university hospital covering the south-west of the Netherlands, an area with approximately 4 million inhabitants. The hospital serves as a third referral centre for patients with benign and malignant esophageal disorders. This includes referral of patients suspected to suffer from achalasia for diagnostic work-up, treatment, and follow-up. Since 1975 all patients referred with such a suspicion of achalasia were evaluated and treated by a selected number of physicians according to a strict protocol, which did not change during follow-up (see below). Every patient diagnosed with primary achalasia in the period of 1975 to 2006 was included in this study.

The diagnostic process included a medical history with special attention to duration of symptoms, physical examination, esophageal manometry, timed barium esophagram and upper gastrointestinal endoscopy.

Treatment

Once the diagnosis of primary achalasia was established, all patients with substantial complaints (in general Eckardt score of three or more) were offered treatment. The Eckardt score consists of three items: dysphagia, regurgitation, and chest pain. For each item a score of 0 (none), 1 (occasional), 2 (daily), or 3 (each meal) is made generating an overall score between 0 and 9 points[24]. From 1974 till 1976, homemade pneumodilators were used. Since then, dilation balloons became commercially available and these procedures were carried out with the use of a Rigiflex balloon (Boston Scientific, Natick, MA, USA).

Baseline dilatation was performed on 3 consecutive days with balloons of either the same or incremental (30, 35, and 40 mm) diameter. Under conscious sedation the balloon was positioned fluoroscopically at the gastro-esophageal junction and inflated to a pressure of 300 mm Hg for 1 minute.

Recurrences were treated by pneumatic dilatation or in case of an early or repeated recurrence by laparoscopic myotomy combined with a fundoplication.

Surveillance

Every patient was offered surveillance according to the same protocol. This follow-up protocol was approved by the local institutional review board, patients consented to inclusion and structured follow-up.

Three months after pneumatic dilation, symptoms and body weight were recorded and an esophageal manometry and timed barium esophagram were repeated. These procedures were repeated after one, two, four, and seven years, together with an upper gastrointestinal endoscopy including careful inspection and standardized biopsy sampling (at least 4 specimens taken a few cm above the gastro-esophageal junction and extra samples from suspicious lesions) for detection of inflammation, dysplasia, metaplasia or malignancy. Further follow-up after a 7-years recurrence free interval consisted of a repeated 3-yearly interview and upper endoscopy with biopsy sampling.

When patients were considered too old, suffered from severe co-morbidity or simply refused surveillance endoscopies, they were once yearly followed by telephone-calls to obtain maximal follow-up information. When a Barrett's esophagus was identified (in 8% of the patients) they were offered a Barrett's surveillance protocol.

Statistical analysis

Data on the incidence of esophageal carcinoma for men and women in the general population of the Netherlands could be obtained for the period 1988 until 2006 from the national cancer registry. For the period before 1988 only data from one region in the Netherlands was available; this region did not show substantial differences with the rest of the Netherlands with respect to the incidence of esophageal carcinoma after 1988. The years at risk for every patient in the cohort were defined. Incidences for the population at risk and for the general population were characterized by categorical variables for age class, gender, period and whether it concerned achalasia patients or the general population. The estimated effect for the last, dichotomous, variable was transformed to a hazard ratio. Confidence limits and significance of p-values were chosen to be at the 95% level. To perform this analysis we used S-Plus ® 7.0 for windows, 1988-2005 Insightful Corp (Seattle, WA). The difference in response to dilation therapy between the patients who developed cancer and the patients who didn't develop cancer was computed with a Fisher exact test 2-sided and a chi square test was used to compute differences in food stasis at endoscopy and esophageal widening at barium contrast esophagraphy between the two groups of patients (SPSS 13.0 for windows, Chicago, IL).

RESULTS

Over the period 1975-2006 a total of 448 patients (218 male, mean age 50.9 years) were diagnosed with primary achalasia (table 1).

Two patients presenting with a gastro-esophageal malignancy within the first year of FU after start of symptoms were excluded, based on the likelihood that the dysphagia symptoms were caused by the cancer (secondary achalasia instead of primary achalasia).

In addition three other patients were excluded because they were diagnosed with achalasia and esophageal carcinoma at the same time. At the time of diagnosis they appeared to have had achalasia symptoms for many years. This means that they have no time at risk for

	Esophageal cancer	**Total group achalasia patients**
Number of patients	15	448
Gender M:F (n)	10:5	218:230
Delay (years)	13 (1-36)	5.6 (0-30)
Age diagnosis achalasia	61 (31-87)	51 (4 – 92)
Age diagnosis esophageal cancer	71 (36-90)	
Number of patients receiving Single treatment: repeated treatment	9:6	287:121
Mean follow-up after first symptoms (years)	24 (10-43)	15 (0- 61)
Outcome: (n)		
Lost		94
Death		100
In surveillance		239

Table 1 Patient characteristics

developing cancer while being diagnosed with achalasia. It is possible these patients would never have been diagnosed with achalasia if they didn't develop esophageal carcinoma. Any similar patient not seeking help for achalasia symptoms would not end up in the control group of achalasia patients not developing esophageal cancer.

The mean follow-up was 15 years (range 0.1 – 61) after start of symptoms and 9.6 years (range 0.1 – 32) after diagnosis of achalasia. This large difference in follow-up is related to the time of inclusion in the cohort. By 2006 one-hundred patients (52% women) had died from non-achalasia related causes after a mean follow up of 8 yrs (0-22) following diagnosis and at a mean age of 80 yrs (54-96). A total of 94 patients (56% women) were lost to follow-up after a mean follow-up of 6 yrs (0-22) following diagnosis and at a mean age of 56 yrs (20-88) (table 1).

Four-hundred and eight (91%) patients were treated with graded pneumatic-dilation. Six (1%) patients were operated without previous dilatation, one elderly patient was treated with botulin toxin, and 33 (7%) patients were not treated until end of follow-up. Sixty-eight (15.1

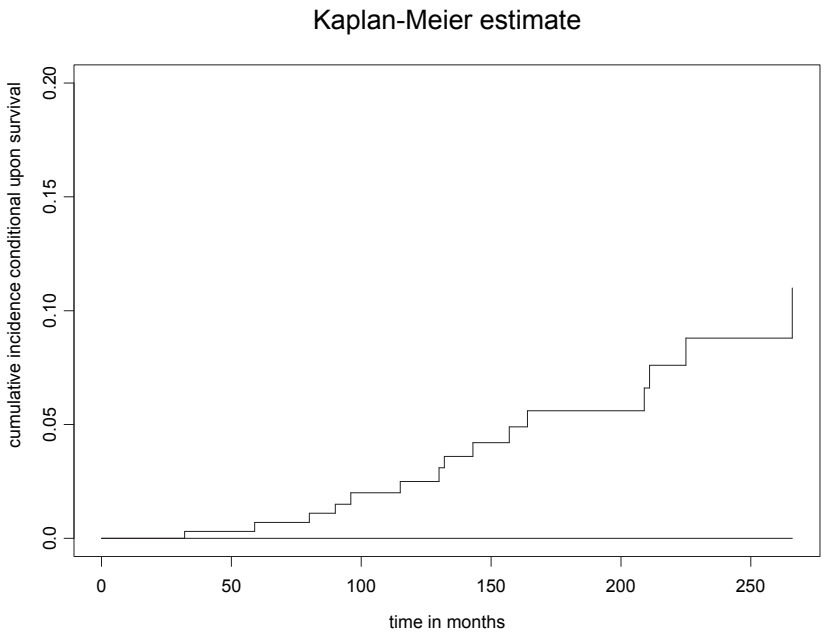

Figure 1: Cumulative incidence of esophageal cancer (y-axis) after diagnosis of achalasia (in months, x-axis). The risk of development of esophageal cancer increased from less than 1% five years after the diagnosis of achalasia to approximately 4% and 10% after 10 and 20 years respectively.

%) patients had received previous treatment before referral but they nevertheless presented with persistent symptoms and were dilated again.

A total of 4,483 person years at risk were observed in this study. The expected rate of esophageal cancer using the incidence figures of the regional cancer registration would be 12 per 100,000 specific for the age, gender and calendar year. Therefore, we could expect 0.54 patients with esophageal cancer in our cohort.

During follow-up, 15 (3.3%) patients (10 men) developed esophageal cancer (3 adenocarcinoma in Barrett's metaplasia and 12 squamous cell carcinoma). The mean age at initial presentation with achalasia was 61 years (range 31-87) after a mean duration of symptoms of 13 years (range 1-36). The mean age at diagnosis of esophageal cancer was 71 years (range 36 – 90). Esophageal cancer was diagnosed a mean of 11 years (range 2 – 23) after initial presentation and a mean of 24 years (range 10 – 43) after symptom onset (table 1). The Kaplan Meier curve (Figure 1) shows the development of cancer after achalasia diagnosis.

The observed incidence rate was 335 (confidence interval 202 - 555) per 100.000 person years. The hazard ratio for achalasia patients to develop esophageal cancer was 28 (confidence interval 17 - 46) in our cohort.

All cancer patients were Caucasian. Only two of them had a history of smoking, none of them had a history of alcohol abuse, nor had any of them suffered from lye ingestion. The

prevalence of esophageal dilatation at baseline recorded with timed barium esophagram was equal in the patients who developed cancer compared with the controls computed with a chi-square test (p= 0.630). Also food stasis recorded at the first endoscopy was no predictor for cancer risk during follow-up (p = 0.299).

Medication use was also studied but was no predictor for cancer risk, which can among others be related to the fact that individual drugs were often prescribed for a limited period and medication changes occurred frequently.

In our total cohort, 278 (71 %) patients had a persistent remission after one series of three pneumatic dilations. Of the 15 patients with esophageal carcinoma, treated with pneumatic dilatation, pneumatic dilation had been successful in 9 patients (60 %, p=0.39, n.s. (Fisher's exact test two-sided)), 5 had needed a second series of dilations and one a third.

At the time of cancer diagnosis, ten patients were still under endoscopical surveillance. In five patients, the surveillance had been stopped at an earlier stage for reasons of age, co-morbidity, or patient preference; they were interviewed annually by telephone. All esophageal carcinomas were diagnosed in targeted biopsy samples.

Five patients (all diagnosed with cancer during a surveillance endoscopy) were treated with esophageal resection. Following surgery, two patients had a long disease-free survival (>8 and >10 years), one patient died 4 years later due to a non-related disorder, and one patient developed metastases 6 years later. The fifth patient died of recurrent cancer within 2 years after resection. The remaining 10 patients received palliative care because they were diagnosed with advanced disease (n=6), or had severe co-morbidity precluding surgery (n=4).

DISCUSSION

The first relation between achalasia and esophageal carcinoma was reported in 1872. Since then, there have been several case-reports and studies conducted on this subject with controversial results against a background of often small patient numbers and limited length of follow-up. Esophageal cancer may arise as a result of chronic food stasis leading to chronic inflammation, epithelial hyperplasia, multifocal dysplasia and eventually squamous cell carcinoma (SCC) [2, 25, 26]. An alternative pathway may occur after adequate dilation therapy of the lower esophageal sphincter, provoking gastro-esophageal reflux, Barrett esophagus (BE) and adenocarcinoma (AC) [5, 6, 27]. Either of these routes may take decades. Therefore malignancies tend to occur no sooner than 15-20 years after first achalasia symptoms, and studies with shorter follow up may underestimate the malignancy risk. This estimate was in many studies further challenged by reports on follow-up from time of diagnosis instead of from time of symptom onset, which despite all uncertainty of reporting is likely to be more relevant for the purpose of cancer risk estimates.

Our cohort had a mean follow-up of 15 years (range 0-61) after first achalasia symptoms. The cancer patients in this cohort developed their malignancy after a mean of 24 (10-43)

years after the onset of achalasia symptoms (Table 1). As illustrated by the Kaplan Meier curve for the development of esophageal cancer, the cancer risk increased rapidly with the length of follow-up (Figure 1). The average delay until diagnosis of achalasia in our cohort was 5.6 years (range 0-30), which is not dissimilar from other studies[28] . Three cancer patients (not included in this cohort) in fact only presented when they had developed invasive esophageal cancer and appeared to have a fully decompensated achalasia esophagus and in retrospect had had achalasia symptoms for 24 to 35 years. These cases show that the individual estimate of cancer risk should be based on symptom duration instead of follow-up since diagnosis of achalasia.

We observed 15 cases of esophageal cancer in 448 patients with a total of 4,483 patient-years at risk, which equals an incidence rate of 335 (confidence interval 202-555) per 100,000 per annum (0.34%) and a hazard ratio of 28 (confidence interval 17-46). This equals the annual incidence to develop gastric cancer in case of gastric intestinal metaplasia or atrophic gastritis[29] and is slightly lower than the reported annual incidence of Barrett's carcinoma in Barrett's esophagus of 0.5 – 1.0 %[30]. Surveillance in Barrett's esophagus is generally accepted and surveillance is in some countries advocated for intestinal metaplasia of the stomach and atrophic gastritis. Similarly, surveillance in achalasia could be propagated.

The number of patients lost to follow-up is relatively high. These lost patients were not treated for esophageal carcinoma in our hospital but it is possible that they developed malignancy and were treated in another hospital. The patients who died of other causes also had a shorter follow-up but were relatively old. The total number of years at risk of our cohort was large and allowed a reliable comparison with the general population. As patients lost to follow-up did not differ in any major aspect (age, sex, duration of achalasia, prevalence of esophageal dilatation and food stasis) to those who completed follow-up, there is no reason to assume different esophageal cancer incidence in the lost-to-follow up patients, and thus a different relative cancer incidence.

Apart from disease duration, one may hypothesize that the efficacy of LES dilation therapy may also affect the risk for esophageal cancer in achalasia. Efficacious therapy aims to reduce food stasis, which might interrupt the progression to carcinoma and thus reduce the squamous cell cancer risk. Data about the relationship between dilation therapy and squamous cell cancer risk are however scarce. In our total cohort, 71 % of patients had a persistent remission after one series of three pneumatic dilations. Of the 15 patients with esophageal carcinoma, treated with pneumatic dilatation, pneumatic dilation had been successful in 9 patients (60 %, p=0.39, not significant (Fisher's exact test 2-sided)). Therefore, in our study the efficacy of therapy did not appear to influence the malignancy risk. None of the 15 cancer patients had been treated with a myotomy and/or fundoplication. In total, only 26 patients were operated and therefore our results do not allow any calculation on the potential effect of myotomy of fundoplication on esophageal cancer risks.

Another argument to perform surveillance is the fact that esophageal malignancies in achalasia patients are often detected late and in an advanced stage, because symptoms of obstructive cancer mimic the presenting symptoms of achalasia [21]. However a 3-yearly surveillance could, in our series, not prevent that 6 of ten patients under surveillance (60%) died of esophageal cancer within 2 years. This mortality risk resembles the mortality risk of esophageal cancer in the general population but is in contrast with the reported survival of Barrett's adenocarcinoma diagnosed by surveillance of 73 – 85% [31, 32] . An explanation could be that in Barrett's esophagus the whole segment at risk is relatively small and is more easily mapped by endoscopy and biopsy sampling than in cases with achalasia. Another difference is the difficult inspection in achalasia patients due to mucosal food adherence.

These facts raise the question if long-term surveillance in achalasia is useful and if so how it should be performed. We used a follow-up protocol for early detection of symptom recurrence, reflux esophagitis, as well as progression towards neoplasia. Patients underwent manometry, timed esophagraphy and endoscopy with biopsy sampling 1, 2, 4 and 7 years after pneumatic dilation and after 7 years at least every 3 years. In case of dysplasia or severe inflammation the interval was shortened. During every endoscopy random biopsies of the distal esophagus were taken together with targeted biopsies from mucosa with suspect appearance. Probably surveillance may possibly be improved by the use of specific histological markers [33] or the aid of lugol staining [34] The surveillance time interval of three years is chosen at the beginning of the protocol in 1975 and was based on the limited data of esophageal carcinoma in achalasia and the lack of data on the effect of surveillance on survival.

A last argument against surveillance is cost efficacy. The low cancer incidence requires many investigations to detect one cancer, and nevertheless many of these malignancies remain to have a very poor prognosis, together leading to high costs per life-year saved.

Therefore, when combining the pros and cons it seems useful to define high risk patients and to develop a tailor-made surveillance program which starts 10 years after first achalasia symptoms, with a shorter interval than the 3 years used in our series and probably a different technique. Known risk factors for squamous cell cancer such as male gender, age over 60 years, cigarette smoking, and alcohol use probably should be taken into account and perhaps advance surveillance to earlier years. In the cohort a very low number of patients smoked (13%) or drank more than 2 units alcohol a day (2%). This prevented us from finding any relation between smoking and alcohol intake on esophageal cancer incidence in our cohort.

To enhance the accuracy of endoscopic surveillance, patients can be kept on a liquid diet for 24 to 48 hours prior to endoscopy and be treated with additional esophageal lavage if food retention or stasis compromises a clear view at endoscopy. These issues and the use of histological markers in the biopsy samples, as well as more advanced endoscopic imaging methods need to be further studied as esophageal cancer remains an important threat for patients with long existing achalasia.

CONCLUSION

The relationship between achalasia and esophageal cell carcinoma is established with an incidence rate of 0.34 % per year of follow up. This annual incidence rises with length of follow-up and is much higher after 20 years of follow-up (Figure 1). Although the absolute risk is low this is significantly increased compared to the general population (hazard ratio 28). The prognosis of patients with achalasia and esophageal carcinoma remains poor, even when they were under regular endoscopic surveillance. To improve this prognosis, we should define high-risk achalasia patients and keep them under more frequent, probably annual endoscopic surveillance starting 10 years after symptom onset. In combination with known other risk factors the surveillance may be started earlier. More studies are needed to establish the optimal screening interval and technique.

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Chapter 5 Barrett's metaplasia

Barrett's esophagus and esophageal adenocarcinoma are common after treatment for Achalasia

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ABSTRACT

Background: Achalasia is characterized by esophageal a-peristalsis and impaired relaxation of the lower esophageal sphincter (LES). This contrasts with an insufficient LES, predisposing to gastro-esophageal reflux and Barrett's esophagus (BE). The co-incidence of achalasia and BE is rare. Pneumatic dilatation (PD) may lead to gastro-esophageal reflux, BE development and esophageal adenocarcinoma.

Aims: To determine the incidence of BE and esophageal adenocarcinoma in achalasia patients treated with PD.

Methods: We performed a single-center cohort follow-up study of 331 patients with achalasia treated with PD. Mean follow-up was 8.9 years consisting of regular esophageal manometry, timed barium esophagram and endoscopy.

Results: Twenty-eight (8.4%) patients were diagnosed with BE, one at baseline endoscopy. This corresponds with an annual incidence of BE of 1.00 % (95% CI 0.62-1.37). Hiatal herniation was present in 74 patients, 21 developed BE compared to 7 of 257 patients without a hiatal hernia. Statistical analysis revealed a hazard ratio of 8.04 to develop BE if a hiatal hernia was present. Post-treatment LES pressures were lower in patients with BE than in those without (13.9 vs. 17.4 mm Hg; p=0.03). Two (0.6%) patients developed esophageal adenocarcinoma during follow-up.

Conclusion: BE is incidentally diagnosed in untreated achalasia patients despite high LES pressures, but is more common after successful treatment especially in the presence of hiatal herniation. Patients treated for achalasia should be considered for GERD treatment and surveillance of development of BE, in particular, when they have low LES pressures and a hiatal herniation.

KEYWORDS:

achalasia, Barrett's esophagus, gastro-esophageal reflux, esophageal adenocarcinoma, hiatal hernia.

INTRODUCTION

Achalasia and Barrett's esophagus are generally thought to be the opposing ends of the spectrum of esophageal disease, although both relate to malfunctioning of the lower esophageal sphincter (LES). Achalasia is a rare neuro-degenerative disorder with an annual incidence of 1 per 100,000 inhabitants [1]. It is characterized by a-peristalsis of the esophageal body and high basal LES pressure without a swallow-induced relaxation of the LES. Predominant symptoms of achalasia are dysphagia and regurgitation, often accompanied by weight loss.

In contrast, Barrett's esophagus is a more common condition with increasing prevalence up to 1.6 percent [2-5]. Barrett's esophagus is characterized by the replacement of normal squamous cell epithelium by specialized intestinal columnar epithelium. Patients with Barrett's esophagus have an increased risk to develop esophageal adenocarcinoma [6]. Although the exact etiology of Barrett's metaplasia is incompletely understood, there is a clear relationship with gastro-esophageal reflux disease (GERD) [7]. In contrast to achalasia, GERD is associated with hiatal hernia, low resting pressure of the LES and an increased frequency of transient LES relaxations, both facilitating the reflux of acid gastric contents [8].

Treatment of achalasia aims at reducing LES pressure. This is generally achieved by surgical myotomy or pneumatic dilatation (PD) [9]. The effect of PD treatment depends on age, LES pressure three months after dilatation, obliteration of the balloon waist during dilatation and symptoms of classic achalasia [10]. Effective therapy resulting in a low LES pressure may however lead to GERD, which in theory may predispose to Barrett's esophagus. Few data exist on the incidence of GERD after treatment of achalasia, both because of the lack of performance of specific diagnostic procedures such as 24-hr pH monitoring after achalasia treatment and limited cohort follow-up data in achalasia patients. However, GERD has been reported in 11-25% of achalasia patients following myotomy and in 2% following PD [11]. Even fewer data exist on the development of Barrett's esophagus in patients who underwent treatment for achalasia.

We hypothesized that adequate PD treatment for achalasia may be complicated by the development of Barrett's esophagus and possibly esophageal adenocarcinoma. We therefore studied the incidence of and risk factors for Barrett's esophagus and the development of esophageal adenocarcinoma in a large cohort of achalasia patients treated with PD.

METHODS

Patients

Since 1975 all patients with achalasia referred to our hospital have been treated and followed according to a fixed protocol as described before [10]. The diagnosis and treatment protocol did not change over time and was performed by a limited number of physicians. The diagnostic process included the medical history and physical examination, esophageal manometry, a

timed barium esophagram and an upper GI-endoscopy. During manometry a mean resting LES pressure was recorded as a mean of four (end-expiratory) measurements. LES relaxations and peristalsis of the esophageal body were studied. A timed barium esophagram was performed after swallowing 200 ml barium contrast with standardized records after 0, 1 and 10 minutes. An upper GI endoscopy was done to rule out other causes of dysphagia. Signs of gastro-esophageal reflux disease were noted. A hiatal hernia was diagnosed during insertion and only when the distance between the gastro-esophageal junction and the aperture of the diaphragm was more then 2 cm.

Therapy

Once a diagnosis of primary achalasia had been established or confirmed, all patients were offered treatment with pneumatic dilatation on three consecutive days with an increasing balloon diameter of respectively 30, 35, and 40 mm. The balloons were in the very early days home-made, with a balloon inside a linen cuff with a length of 12 cm and a fixed diameter of 30, 35 and 40 mm. In later years Rigiflex pneumatic dilatation balloons (Boston Scientific, Natick, MA, USA) were used. Dilatation was performed under conscious sedation and fluoroscopic control up to a pressure of 300 mm Hg. All pneumatic dilatations were over the years performed by a total of 3 endoscopists, experienced in pneumatic dilatation.

Follow-up

Every patient was followed according to a fixed protocol. Three months after pneumatic dilatation, patients were interviewed for esophageal complaints, patient's weight was recorded, and esophageal manometry and barium swallow were repeated. This evaluation including upper gastrointestinal (GI) endoscopy was repeated after 1, 2, 4 and 7 years.

Upper GI endoscopy included random biopsy (3-4 biopsies) sampling from the distal esophagus just above the gastro-esophageal junction and targeted biopsy sampling from any specific lesion, such as suspected Barrett's epithelium, dysplasia or malignancy. For histological examination, 4 µm haematoxylin-eosin stained routine histological sections were used. In case of suspected Barrett's esophagus, four biopsies were obtained (one from each quadrant) of every 2 cm of columnar epithelium to assess the presence of intestinal type columnar epithelium. Esophagitis was diagnosed both endoscopically graded according the Los Angeles classification (grade A-D) and histologically. Barrett's esophagus was endoscopically classified as short segment (<3 cm) or long segment Barrett's esophagus. Hiatal hernia was assessed during introduction of the endoscope with limited inflation, and diagnosed when the distance between the gastro-esophageal junction (GEJ) and the aperture of the diaphragm was more than 2 cm.

Further follow-up after 7 years consisted of an interview and upper GI endoscopy with biopsy sampling at least every 3 years. Some patients refused these interval endoscopies or other long-term follow up. These patients were contacted by telephone every year to check

on symptoms and weight loss. In case of symptom recurrence or persistence, patients were either retreated with pneumatic dilatation or underwent surgical myotomy, depending on the time of relapse, success of initial treatment and patient's preferences[10]. If a patient had died, the cause of death was checked with the general practitioner or the civil registration.

Statistics

The development of barrett's esophagus and hiatal hernia were expressed as barrett's-free and hiatal hernia-free survival curves, calculated by means of the kaplan meier method. The uni-variate effect of patient characteristics are given as hazard ratio's estimated by cox proportional hazards analyses together with the log-likelihood p-values. Statistically significant variables, as well as other clinically relevant variables (age, gender) were included in a multivariate cox's proportional hazards analysis. By means of step-wise backward elimination, a final model was constructed comprising variables which were significantly and independently (i.e. controlled for other variables) related to the endpoints barrett's esophagus or hiatal hernia, respectively. As a hiatal hernia event during follow-up often was followed by development of barrett's epithelium, the effect of hiatal hernia on barrett-free survival was included in the cox's proportional hazard model as a time-dependent covariate. For all cox models, the assumption of proportional hazards was investigated for each variable by studying the ln(-ln) plot. All analyses were carried out in spss for windows, version 11.0.1 (spss, chicago, il). The level of statistical significance was set at a two-sided $p<0.05$. LES-pressures were measured during esophageal manometry before and after treatment, the wilcoxon signed rank test was applied to compare these.

RESULTS

Therapy

Over the period of 1975-2003, 394 patients were referred to our hospital with a clinical suspicion of achalasia. Fourteen patients were diagnosed with secondary achalasia caused by malignancy, 16 patients had a non-specific motility disorder, 5 patients had diffuse esophageal spasms and in 10 patients no specific diagnosis was made. The remaining 349 patients were diagnosed with primary achalasia. Six (2%) of these 349 patients refused treatment for various reasons, another 6 were treated elsewhere, 1 preferred surgery and in 5 patients severe co-morbidity prohibited any treatment.

The remaining cohort consisted of 331 patients (male/female 160/171, mean age at diagnosis 51 years, range 4 to 90 years). They were followed for a median period of 8.9 years (range 3 months to 25 yrs). Sixty (18%) patients had received previous treatment (pneumatic dilatation or myotomy (n=19)) before referral but they nevertheless presented with persistent symptoms and were offered dilatation again.

All 331 patients were initially treated with PD. After this initial treatment 241 (73%) patients had a persistent remission of symptoms, whereas 88 (27%) patients were re-dilatated and 3 (1%) patients were treated with myotomy because of symptom persistence or recurrence. Eventually during long follow up, another 13 (4%) patients underwent surgical myotomy after repeated dilatation. Of these 16 operated patients, 6 again had symptom recurrence for which treatment with pneumatic dilatation was applied. The majority of patients were treated immediately after the establishment of the diagnosis of achalasia.

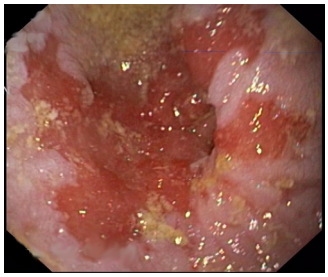
Most patients underwent post-treatment endoscopy, 18 (5%) patients refused routine follow-up endoscopy according to the protocol. During follow up 86 (26%) patients died. In total, 14 (4%) patients developed esophageal cancer, these included 12 patients with squamous cancers and 2 patients with adenocarcinoma. One of these patients was treated by esophagectomy and has since then a long disease free survival. The other patient first was diagnosed with high grade dysplasia for which she was treated with endoscopic mucosal resection followed by ablative therapy (photodynamic therapy). This patient developed an adenocarcinoma with hepatic metastases 6 years afterwards. Sixty-four (19%) patients were lost to follow up after a mean follow up of 6 years (range 0- 19) after PD.

Development of erosive esophagitis

Data on esophageal histology and endoscopy have been published earlier[12]. In brief 66% of patients did not develop any endoscopic signs of esophagitis during follow-up. Grade A esophagitis occurred in 22 % of the patients and 11 % was diagnosed with grade B-D esophagitis according to the Los Angeles classification system at any time during the follow-up. Histological examination however showed a higher prevalence of esophagitis.

Development of Barrett's esophagus

Twenty-eight (8.4%) of the 331 patients were diagnosed with Barrett's esophagus (picture 1). This group consisted of 12 males and 16 females, all Caucasian. Their mean age when diagnosed with a Barrett's esophagus was 55.9 years (range 35-84 years). One patient was diagnosed with Barret's esophagus at baseline endoscopy, whereas new development of



Picture 1: endoscopic picture of a dilated and elongated esophagus with some food retention and histologically proven Barrett's esophagus with high grade dysplasia.

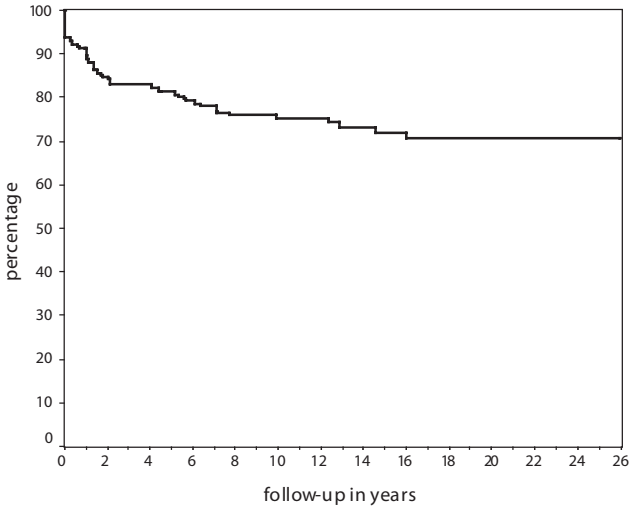


Figure 1: Development of Barrett's esophagus after the diagnosis of achalasia, shown as Barrett free survival in years of follow-up

	Total	Barrett +	Barrett -	HR* (95% C.I.)	p-value
Number	331	28	303		
Female	161	16	145	1.09 (0.51; 2.32)	0.83
Age (yr) mean (S.D.)	51.1 (21.4)	55.9 (15.2)	50.7 (21.8)	1.02 (1.00; 1.04)	0.06
Myotomy	35	7	28	2.5 (1.00; 6.19)	0.07
Pre LES-pressure (mm Hg) median (range)	30.0 (5-207)	30.0 (10-100)	30.0 (5-207)	HR≤30 = 1 HR>30 = 1.06 (0.56; 2.44)	0.90
Post LES-pressure (mm Hg) median (range)	15.0 (2-125)	13.5 (5-35)	15.0 (2-125)	HR≤15 = 1 HR>15 = 0.46 (0.18; 1.14)	0.07
Hiatal hernia present	14	13	1	8.04 (3.58; 18.1)#	<0.001

Table 1 Characteristics of patients with and without Barrett's esophagus and the corresponding Relative Risk's

* Hazard Ratio (HR) estimated by univariate Cox regression analysis of the 27 cases with Barrett during follow-up. The log-likelihood p-value is reported.

HR of hiatal hernia entered as a time-dependent covariate in the Cox regression analysis.

Barrett's metaplasia was observed in the other 27 Barrett's patients. The remaining 27 (8.2%) patients developed a Barrett's esophagus during follow-up at a mean interval of 67 months (range 6 to 224) after initial treatment with pneumatic dilatation. This corresponds with an annual incidence of Barrett's esophagus in this population of 1.00 % (95% CI 0.62-1.37) (Figure 1). Nineteen patients had a Barrett's esophagus less than 3 cm in length, the other nine had a long segment varying in length from 3 to 7 cm. The characteristics of patients with and without Barrett's esophagus are listed in Table 1. Both groups did not differ with

respect to age or sex. Barrett's esophagus tended to be more common after myotomy, but this difference was not significant (RR = 2.5 95% C.I [1.00; 6.19] (p = 0.07).

Development of esophageal adenocarcinoma

During follow-up, 2/28 (7%) patients with a Barrett's esophagus developed esophageal adenocarcinoma. This occurred 25 and 27 years after the initial diagnosis of achalasia, at an age of respectively 70 and 84 years. Both patients had started with endoscopic surveillance from the time of first balloon dilation onwards for a period of respectively 18 and 14 years. In these patients, the development of esophageal carcinoma was not preceded by the finding of dysplasia in the random surveillance biopsies, the last of which had been obtained one respectively two years prior to cancer diagnosis.

Hiatal hernia

A hiatal hernia was present in 74 (22%) of the 331 patients. Sixteen patients were diagnosed with a hiatal hernia prior to PD treatment, the remaining 58 patients after PD treatment.

Twenty-one (28%) patients with a hiatal hernia developed a Barrett's esophagus, compared to 7 (2%) of the 257 patients without a hiatal hernia. Three of the 21 patients with hiatal hernia and Barrett's esophagus had a hiatal hernia at the time of diagnosis of achalasia,

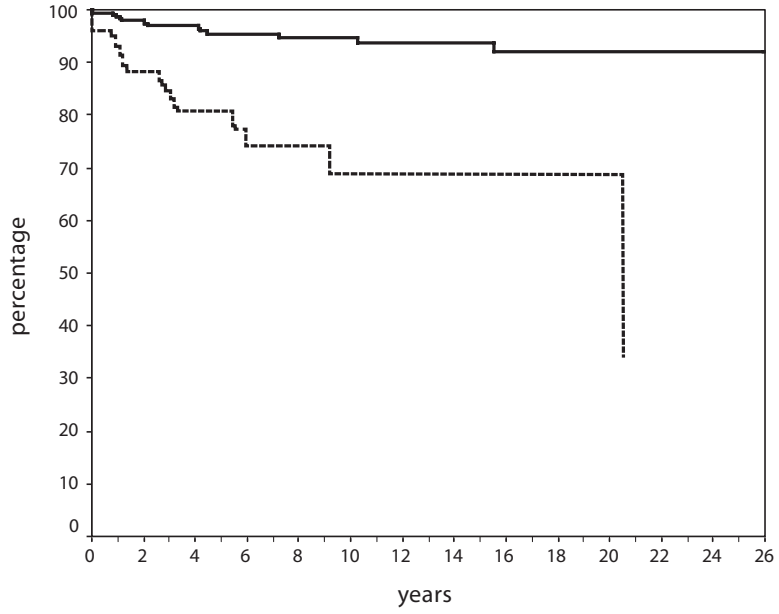


Figure 2 Barrett free survival if no development of hiatal hernia (solid line, time is years since diagnosis of achalasia) and after hiatal hernia (broken line, time is years since development of hiatal hernia)

the remaining 18 patients developed a hiatal hernia at a mean of 6 (range 1-15) years after PD treatment. Statistical analysis using a time-dependent Cox regression analysis revealed a Hazard ratio of 8.04 (95 % CI 3.5-18.1), p<0.001 to develop a Barrett's esophagus if a hiatal hernia is present (Figure 2). In a uni-variate Cox regression analysis older age appeared a significant factor in the development of hiatal hernia, in contrast with sex, myotomy and LES pressure. Statistical analysis of the correlation between Barrett's esophagus and the later development of hiatal hernia also showed significance (HR 8.8, 95% CI 4.32 to 17.9, p<0.001). The results are listed in Table 2.

LES pressures

LES pressures were measured before and after treatment. As expected for the total group of achalasia patients, post-treatment LES pressures were lower than pre-treatment LES pressures (median 15.0 vs. 30.0 mm Hg; p<0.001, Wilcoxon Signed Rank test) indicating effective

	Total	Hiatal Hernia Yes	Hiatal Hernia No	HR* (95% C.I.)	p-value
Number	331	74	257		
Male	160	39	121	1	
Female	171	35	136	0.76 (0.47; 1.21)	0.24
Age (yr) mean (S.D.)	51.1 (21.4)	59.9 (17.2)	48.6 (21.8)	1.03 (1.02; 1.04)	<0.001
No Myotomy	296	63	233	1	
Myotomy	35	11	24	1.6 (0.84; 3.03)	0.18
Pre LES-pressure (mm Hg) median (range)	30.0 (5-207)	30.0 (5-100)	30.0 (7-207)	HR≤30 = 1 HR>30 = 1.09 (0.66; 1.78)	0.74
Post LES-pressure (mm Hg) median (range)	15.0 (2-125)	13.0 (5-70)	15.0 (2-125)	HR≤15 = 1 HR>15 = 0.85 (0.50; 1.43)	0.53
Barrett absent at time of HH	315	65	250	1	
Barrett present	16	9	7	8.80 (4.32; 17.9)#	<0.001

Table 2 Characteristics of patients with and without Hiatal Hernia and the corresponding Hazard Ratio's (HR)

* HR estimated by univariate Cox regression analysis of the 70 cases with Hiatal Hernia during follow-up (the information of the date of Hiatal Hernia were missing in 4 cases). The log-likelihood p-value is reported.

HR of Barrett entered as a time-dependent covariate in the Cox regression analysis.

dilatation treatment. The LES pressures before or after treatment were not significantly related to development of a hiatal hernia.

Patients with a post-treatment basal LES pressures lower or equal to 15 tended to be at higher risk to develop Barrett's esophagus: HR= 2.17 95% C.I. [0.88; 5.56], p=0.07 (Table 1).

Finally, a multivariate Cox's proportional hazards analysis on the development of Barrett's esophagus and hiatal hernia was performed with all clinically relevant variables as age, gen-

	Barrett HR* (95% C.I.)	p-value	Hiatal Hernia HR* (95% C.I.)	p-value
Age (yr) mean (S.D.)		ns	1.03 (1.02; 1.05)	<0.001
No Myotomy	1			
Myotomy	3.49 (1.36; 8.93)	0.009		Ns
Post LES-pressure (mm Hg) ≤15	1			
Post LES-pressure (mm Hg) >15	0.35 (0.13; 0.96)	0.04		Ns
HH / Barrett absent at time of Barrett/ HH	1		1	
HH / Barrett present at time of Barrett/ HH	7.26 (3.19; 16.6)#	<0.001	6.10 (2.87; 13.0)#	<0.001

Table 3 Result of the multiple Cox regression analyses of the endpoints Barrett and Hiatal Hernia. The log-likelihood p-value is reported.
HR of covariate entered as a time-dependent covariate in the Cox regression analysis.

der, therapy, LES-pressures, and hiatal hernia included (Table 3). Age was a significant factor involved in the development of hiatal hernia, but not in Barrett's esophagus. In contrast, myotomy and a post-PD LESP < 15 mm Hg were significantly related to the development of Barrett's esophagus, but not related to hiatal hernia.

DISCUSSION

To our knowledge, only a limited number of patients have been described with the combination of achalasia and a Barrett's esophagus [13-15]. This includes a few patients who developed Barrett's esophagus after pneumatic dilatation [14], 7 patients who were diagnosed with Barrett's esophagus without being treated for their achalasia [14, 16], and 31 patients who had undergone surgical myotomy many years prior to the detection of Barrett's esophagus [14, 15]. Six (16%) of these 39 patients were simultaneously or later diagnosed with esophageal adenocarcinoma.

The incidence of esophageal adenocarcinoma in patients with Barrett's esophagus is considered to be approximately 0.5% per year of follow-up [17, 18] Therefore, the estimated 16% prevalence of esophageal adenocarcinoma in patients with achalasia and Barrett's esophagus as deducted from this figures above seems high.

This is the first study that systematically investigated the relationship between achalasia treated with pneumatic dilatation, Barrett's esophagus and esophageal adenocarcinoma. We observed that 8.4% of 331 patients with achalasia developed a Barrett's esophagus, in a single case prior to treatment, but mostly after dilatation treatment. Risk factors associated with the development of Barrett's esophagus were the presence of a hiatal hernia, prior myotomy and a lower LES pressure. During follow-up, 2/28 (7%) patients with Barrett's esophagus developed an esophageal adenocarcinoma.

Myotomy was performed in 35 patients, in 19 patients this had occurred before referral, yet these 19 patients received renewed pneumatic dilatation for recurrent symptoms after

surgery. Another 16 patients were treated with myotomy during FU, 6 of them received additional dilatation after surgery. Both treatment modalities have the same goal, namely disrupting the LES muscle fibers. A recent study could not show a superiority in success rate of myotomy [19]

The combination of achalasia and Barrett's esophagus is considered to be rare as only 39 cases of achalasia with Barrett's esophagus have been reported in the literature mostly after myotomy [14, 15]. Although Barrett's esophagus and achalasia seem to be disorders at the opposing ends of a spectrum, our results show that there is an association between treatment of achalasia, leading to an insufficient LES, and the development of a Barrett's segment. In our population, the prevalence of Barrett's esophagus in achalasia patients after treatment was 7.4 %, with an annual incidence of newly development of Barrett's esophagus of 1.0 % corresponding with a 1000 new cases per 100,000 subjects / year.

These findings contrast with data on the prevalence and incidence of Barrett's esophagus in the general population. Endoscopy and autopsy series have suggested a prevalence of 1 % in Western populations [20]. This prevalence may however be higher in selected populations. One study for instance reported a 25 % prevalence of Barrett's esophagus in a population of male veterans [21]. Estimates for the number of new cases per year vary between 10 and 48 per 100,000 inhabitants [22, 23]. We recently observed an annual incidence of 24.7 per 100,000 / year in a Dutch population of 386,000 subjects followed for 3.4 years [2]. In conclusion, our data suggest that patients treated for achalasia have a higher risk of developing Barrett's esophagus than the general population. As there is no age and sex comparable control population in our study of course there is surveillance bias. In the absence of such a population, we can only make an indirect comparison with other data. Klinkenberg-Knol et al observed that 20 (12%) of 166 patients with severe reflux disease treated with omeprazole developed Barrett's metaplasia during an average 6.5 years follow-up with annual endoscopy [24]. This incidence is in the same order of magnitude as our observation of Barrett's metaplasia in 7.3% of our achalasia patients during an average 8.9 years follow-up.

Several hypotheses may explain the association between achalasia and Barrett's esophagus. The most obvious hypothesis is that efficient treatment leads to insufficiency of the LES predisposing to GERD and possibly to its complications[25]. This is supported by the association between a lower LES pressure and the development of Barrett's esophagus in our cohort and also by our findings that esophagitis is very common during follow-up after PD [12].

Although all achalasia patients were at each visits asked for esophageal complaints it can be difficult to discriminate symptoms related to the primary motility disorder and those related to the secondary reflux after treatment. Since most achalasia patients have persistent esophageal complaints we were unable to find a clear correlation between classical reflux symptoms and the development of Barrett's esophagus after achalasia treatment.

Surprisingly in literature and also in our study a few achalasia patients appeared to have Barrett's esophagus already prior to baseline treatment. One hypothesis is that the develop-

ment of Barrett's esophagus had already occurred before achalasia started. Secondly it has recently been shown that transient LES relaxations (TLESR's) accompanied by acid reflux may also occur in achalasia patients[26]. The combination of TLESR-induced acid reflux with impaired esophageal clearance may therefore be an explanation for the observed development of Barrett's esophagus in both treated and untreated achalasia patients. A last explanation could be that in (untreated) achalasia patients chronic food stasis and fermentation of retained food may cause chronic esophageal inflammation, predisposing to Barrett's esophagus [16, 27 20, 28 29, 30].

Apart from TLESR's as a predisposing mechanism to reflux, the presence of a hiatal hernia is also an important etiologic factor in the development of GERD and Barrett's esophagus. Various studies have reported a prevalence of hiatal hernia in 95% of patients with severe reflux and Barrett's esophagus with a length of 3 cm or more and 74% in shorter Barrett's esophagus [3, 31, 32]. We found that 28% of patients with a hiatal hernia developed BE in contrast to only 3% of achalasia patients without a hiatal hernia and the presence of a hiatal hernia after treatment of achalasia revealed a hazard ratio of 8.04 to develop Barrett's esophagus. We were surprised to find that 74 out of 331 (23%) achalasia patients had a hiatal hernia. Previous cross-sectional studies in achalasia have reported a prevalence varying between 1.4 % and 2.3 % [33-35]. Goldenberg et al described a higher prevalence, i.e., 14.1 % hiatal hernias in achalasia probably because radiographic examinations were reviewed [36]. They found that 8 of 10 radiographically demonstrated hiatal hernias had not been recorded in the clinical records proving underreporting probably because of the seemingly triviality of the diagnosis. The assessment of hiatal hernias poses some difficulties. Both barium swallow and upper GI endoscopy in particular have limited sensitivity in diagnosing small herniation [37, 38]. High resolution manometry may be the most accurate instrument to diagnose these small herniations. We assessed the presence of a hernia during insertion of the scope and also used the retroflection view to grade the severity of the hernia and only diagnosed a hiatal hernia if the length was more than 2 cm.

Our results confirm that a hiatal hernia is not uncommon in achalasia patients and show that it is a significant risk factor for the development of Barrett's esophagus after esophageal dilation (Figure 2).

Barrett's esophagus is a pre-malignant condition predisposing to esophageal adenocarcinoma. The esophageal cancer risk in patients with a Barrett's esophagus differed between 1/52 to 1/297 years of follow-up in different reports[17]. The incidence of adenocarcinoma in our achalasia patients with Barrett's esophagus was 7.1% (2/28) during an average of 13.4 years follow-up, which corresponds to previously mentioned annual risk of 0.5% [14]. Reviewing all the cases of achalasia combined with Barrett's esophagus, Guo et al found that adenocarcinoma had occurred in 19 % of achalasia cases with Barrett's esophagus, that developed a mean of 18 years after treatment for achalasia [14]. The mean follow-up of the Barrett's patients in our study was 13.4 years in total after achalasia diagnosis and 7.5 years after Barrett's de-

velopment. With longer follow-up, the incidence of Barrett's esophagus and adenocarcinoma in our cohort may further increase. The presence of Barrett's esophagus could have alerted both patients and physicians, but we found no significant difference (p=0.4) in drop-out percentage between Barrett and non-Barrett patients.

CONCLUSION

Patients with achalasia treated with pneumatic dilatation are at considerable risk for development of Barrett's esophagus. We observed the development of Barrett's esophagus in 28 (8.4%) of 331 achalasia patients before treatment and during long-term follow-up. This implicates that achalasia and Barrett's are not mutually exclusive disorders. In contrast, achalasia and LES lowering therapy should be considered a risk factor for the development of Barrett's mucosa. Hiatal hernia and lower esophageal sphincter pressures increases the risk to develop Barrett's esophagus. During endoscopic and radiographic follow-up of achalasia patients, careful attention should be paid to the presence of hiatal hernia especially in combination with low LES pressures, since this combination strongly increases the risk for Barrett's esophagus and eventually adenocarcinoma of the esophagus.

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Chapter 6 Surveillance

Expression of p53 as predictor for the development of esophageal cancer in achalasia patients

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ABSTRACT

Introduction: Patients with longstanding achalasia have an increased risk of developing esophageal cancer. Surveillance is hampered by chronic stasis. We investigated whether aberrant expressions of tumor suppressor gene p53 and proliferation marker ki67 are early predictors for progression to malignancy.

Methods: In 399 achalasia patients, 4% died of esophageal cancer despite surveillance. We performed a cohort study, using surveillance biopsies from 18 patients (11 carcinoma, 1 high grade dysplasia (HGD) and 6 low grade dysplasia (LGD)) and 10 controls (achalasia patients without cancer or dysplasia development). 164 biopsies were re-evaluated and studied for p53 and ki67 expression using immunohistochemistry.

Results: 82% of patients with cancer/HGD showed p53 overexpression in surveillance biopsies at a mean of 6 (1 - 11) yrs prior to cancer development. In 67% of patients with LGD and only in 10% of the controls p53 overexpression was present. The proportion of samples with p53 overexpression increased with increasing grades of dysplasia. We found no difference for ki67 overexpression.

Conclusions: p53 overexpression may identify achalasia patients at increased risk of developing esophageal carcinoma. Further study is needed to determine if patients with p53 overexpression would benefit from intensive surveillance to detect esophageal neoplasia at a potential curable stage.

KEY WORDS

Achalasia, esophageal cancer, surveillance, p53, immunohistochemistry

INTRODUCTION

Achalasia is a motor disorder of the esophagus characterized by aperistalsis of the distal esophagus in combination with absent or impaired relaxation of the lower esophageal sphincter (LES). This causes symptoms of esophageal obstruction. It is an uncommon disorder with an annual incidence of 1 per 100,000 in the Western world and an equal prevalence in men and women with a peak incidence around 60 years of age [1]. Chronic food stasis (often present despite lower esophageal sphincter (LES) pressure lowering treatment) has been suggested to lead to chronic inflammation, epithelial hyperplasia, multifocal dysplasia and squamous cell carcinoma (SCC) [2-4]. On the other hand LES-pressure lowering therapy by balloon dilatation or surgical myotomy may lead to chronic gastro-esophageal reflux, which in some cases is ultimately complicated by the development of Barrett esophagus (BE) and esophageal adenocarcinoma (AC) [5, 6, 7, 8].

Achalasia is considered to be a premalignant disorder. The reported risks for esophageal carcinoma vary widely between different studies. Several autopsy studies have reported an esophageal neoplasia prevalence of over 20% in achalasia patients at the time of death [9-11]. Wychulis et al. conducted the largest cohort follow-up study in 1318 patients with achalasia followed for a mean of 17 years. This study revealed a seven-fold increased risk for esophageal cancer compared to the general population [12]. Other follow-up studies reported esophageal cancer incidences varying from zero per 953 patient-years to 1 per 173 patient-years, which translates into relative risks varying from zero to 140 times increased compared to a sex- and age-adjusted population [13-15].

Surveillance should aim at detection of neoplastic transformation at an early, curable stage, as well as at the identification of patients at the highest risk of developing neoplasia. These are in particular those patients, who have persistent severe inflammation or food stasis despite LES-pressure lowering therapies and those with BE. Unfortunately, surveillance in achalasia is difficult to perform due to stasis and mucosal adherence of food, which compromise a careful inspection. Besides, most carcinomas develop in the middle and distal third of the esophagus and therefore, in contrast to Barrett's esophagus, the whole length of the esophagus should be carefully inspected and sampled [16]. Finally, histological evaluation of esophageal surveillance biopsy samples may be hampered by the persistent presence of chronic inflammation.

For these reasons, markers for the identification of patients at an increased risk of developing malignancy are needed. We therefore investigated whether expression of the tumor suppressor gene p53 and proliferation marker Ki67 are early predictors for progression to malignancy and are able to identify those patients with achalasia who are at highest risk of developing esophageal cancer. These markers were used earlier to predict malignant transformation in Barrett's metaplasia [17, 18].

MATERIAL AND METHODS

Patients

Since 1975 all patients with achalasia referred to our hospital have been treated and followed according to a strict protocol. The total cohort consisted of 399 patients (male/female 198/201), who have been followed for a mean of 8.1 years (0 – 25.9) after diagnosis. The diagnosis and treatment protocol did not change over time and patients were taken care of by a selected number of physicians over the complete period of follow-up of the cohort. All patients were first treated with repeated pneumatic dilatation on three consecutive days using Rigiflex balloons (Boston Scientic, Natick, MA, USA) with increasing diameter (30/35/40 mm). Recurrent symptoms were treated by repeat dilation. Patients with an early (within 3 months) or repeated recurrence (after 3-4 dilation sessions) of symptoms were referred for Heller myotomy in combination with a Dor fundoplication.

Surveillance endoscopies were performed at least every three years. During surveillance a careful inspection of the esophagus was performed and random biopsy samples were taken from the distal part of the esophagus, 1-2 cm above the presumed LES. Suspicious areas were separately sampled. If food stasis compromised clear vision, patients were asked to return after a 3-day liquid diet. Patients who refused surveillance endoscopies or were considered too old to undergo surveillance were followed by phone calls at regular intervals.

Twelve achalasia patients (M/F 7/5) who developed esophageal cancer or high grade dysplasia (HGD) were included in the current study. In addition ten achalasia patients with comparable duration of achalasia, gender and age but without esophageal cancer served as controls. Six patients, who developed low-grade dysplasia (LGD, 3 in BE) during follow-up, were also included in this study.

Histology and immunohistochemistry:

From the patients who developed esophageal carcinoma or dysplasia all available samples and from the control patients the five most recent biopsy samples were re-evaluated for the presence of Barrett's esophagus, dysplastic changes (LGD or HGD) and carcinoma (AC or SCC) by an expert GI-pathologist (H. van Dekken) who was blinded for the clinical information. The available paraffin blocks of these samples were retrieved from the archive and studied for p53 and ki67 expression using immunohistochemistry.

Four µm tissue sections of the formalin-fixed, paraffin wax-embedded samples were sliced and mounted on adhesive slides, dried and deparaffinized with xylene and rehydration through graded ethanol. Antigen retrieval was performed by boiling these samples for 15 minutes in 10 mM monocitric acid buffer (pH 6.0) for ki67 staining and in 10mM Tris/EDTA buffer (pH 9.0) for p53 staining. Prior to immune staining, endogenous peroxidase activity was blocked by incubating the slides in a 0.5 % solution of H_2O_2 in phosphate-buffered citric acid for 15 minutes at room temperature. Samples were 3 times washed with TRIS-buffered

saline (TBS) with a pH of 7.4. The samples were incubated in TBS buffer containing 10% rabbit non-immune serum (DAKO, Glostrup, Denmark) and 10% normal human plasma (and 5% BSA for p53 staining) for 20 minutes. Sections were incubated with the primary antibody anti-human ki67 antigen (clone MIB-1, DAKO) in a 1:100 dilution or p53 antigen (clone DO-7, DAKO) in a 1:100 dilution for 14 hours at 4ºC. Subsequently, biotin-labelled rabbit-anti-mouse antibody (DAKO) was used as second antibody followed by the addition of a streptavidin-horseradish peroxidase complex (DAKO). To detect ki67 and p53, 3-amino-9-ethylcarbazole was used as substrate. Two independent researchers who were blinded to the clinical data counted at least 300 nuclei in every sample. Only longitudinally sectioned squamous and Barrett's epithelium was evaluated. Cells were counted as positive when moderate to intense red nuclear staining was found. An isotype and a negative control staining were performed. Samples were considered p53 and ki67 positive if more than 15% of nuclei were stained (Fig 1). When the counts of both researchers on a specific sample differed more than 10%, both researchers counted the sample again. When there was still at least a 10% difference a third researcher counted the sample for a final classification (negative / positive).

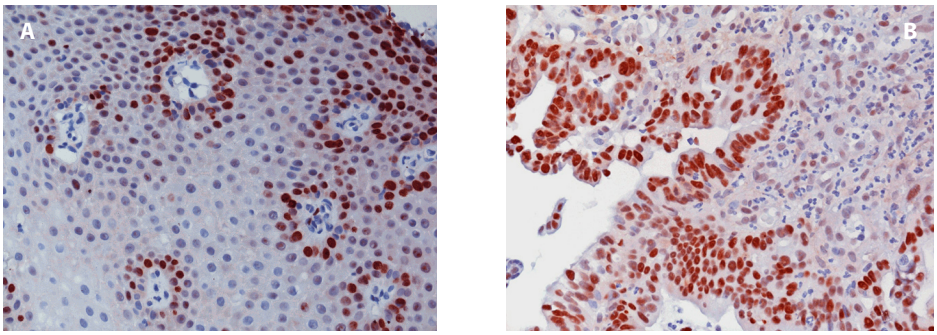

Figure 1: examples of ki67 (A) and p53(B) overexpression in esophageal biopsy samples of patients with achalasia.

Statistics

Statistical analyses were conducted using SPSS software. (SPSS version 13.0, Chicago, IL, USA). Chi-square tests and t-tests were used to analyse the results of ki67 and p53 expression. The hazard ratio to develop carcinoma or high-grade dysplasia was calculated using Cox regression analysis, with and without adjustment for histology, using age as the time-axis and p53 status as a time dependent variable. The time course of p53 expression of the carcinoma patients in time was visualised using cubic spline functions in S-plus 6.0 software (Insightful Inc, Seattle WA).

RESULTS

Patients

From 12 cancer cases (eight SCC, three AC and one HGD), 65 follow-up biopsy samples, obtained prior to the development of malignancy, were available. The mean age of these patients at achalasia diagnosis was 62.5 years (range 33 - 80) and they were followed-up for a mean of 22 years (range 9 - 33) after the onset of achalasia symptoms. One patient, who developed HGD during follow-up and underwent esophageal resection, was included in this group. In addition six patients (mean age at diagnosis 50 years (range 28 - 62) and followed-up for a mean of 20 years (range 5 - 44) who developed (LGD) during follow-up) were also selected and included. Ten patients with a mean age of 46 years (range 17-75) and a mean follow-up of 21 years (range 14 - 28) after start of achalasia symptoms and at least five available samples served as controls. Patient characteristics are listed in Table 1.

	Esophageal cancer/high grade dysplasia patients N=12	**Low grade dysplasia patients N=6**	**Control patients N=10**
Squamous cell ca	8		
Adenocarcinoma	3		
Low grade dysplasia		6	
High grade dysplasia	1		
Barrett's metaplasia	4	3	0
Male/female	7/5	4/2	6/4
Age of diagnosis	63 (33-80)	50 (28-62)	46 (17-75)
Duration of symptoms till carc/ dysplasia/end fu	23 (9-33)	Dysplasia: 11 (4-23) End FU: 20 (5-44)	21 (14-28)
Age of carcinoma/end FU	74 (38-88)	65 (53-76)	63 (30-84)

Table 1: Patient characteristics

Histology

One hundred sixty-four samples from 28 patients were re-evaluated. All three patients with AC who had undergone an average of 5 (range 2 - 8) endoscopies with biopsy sampling during follow-up displayed BE in biopsies taken prior to cancer development, whereas only one patient showed LGD and HGD prior to cancer development.

From eight patients who had undergone an average of 5.4 (range 3 - 10) endoscopies with biopsy sampling during follow-up, who developed SCC, only three patients were diagnosed with LGD or HGD in the prior biopsies. One patient already displayed LGD, one also HGD and in one patient some of previous biopsies showed BE and/or LGD.

In the patient with HGD, this dysplasia developed in previous noticed BE with already LGD in two earlier samples.

Six LGD patients had undergone an average of 6.5 (range 3 - 10 per patient) endoscopies with biopsy sampling during follow-up. Three of them also had BE in the biopsy samples prior to and together with dysplasia development. In three patients LGD was present in some of the consecutive samples, but this was no longer found in samples taken at a later time point during follow-up. The other three patients showed LGD in the more recent samples, obtained at the end of follow-up.

Ten controls had undergone biopsy sampling during their follow-up with an average 5.6 (range 5 - 7 per patient) endoscopies. Dysplasia and metaplasia were not present in any of these samples upon blinded revision.

Ki67 expression

Ki 67 expression was assessed in 135 samples from 28 achalasia patients (Fig 2). Eighteen paraffin blocks were missing in the archive and 14 slides were empty or not assessable due to background staining. Ki67 expression was positive in 45/51 (88.2%) control patient samples, 26/30 (86.7%) samples from dysplasia patients and 47/54 (87.0%) esophageal cancer or HGD

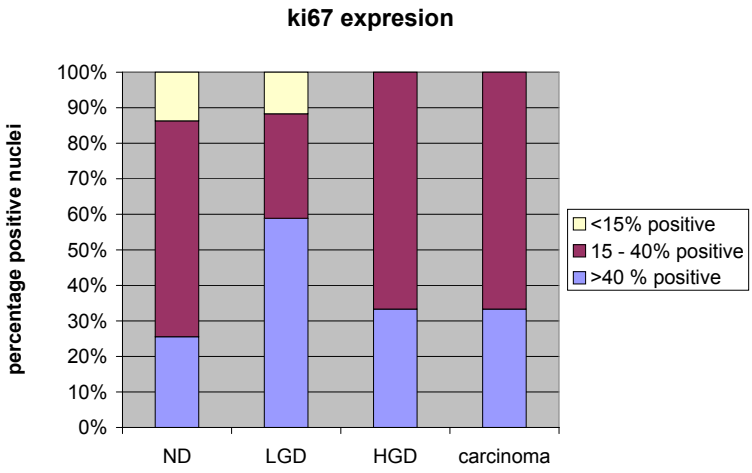

Figure 2: Number of ki 67 positive nuclei in esophageal biopsies without dysplasia (ND), with low-grade dysplasia (LGD), with high-grade dysplasia (HGD) and with carcinoma.

patient samples. Chi-square testing showed no statistically significant differences between these groups (p=0.97). In addition the proportions of positive nuclei were not significantly different between the three groups (87% in cancer and HGD cases, 86.7% in LGD cases and 88.2% in controls); (p=0.38 cancer compared to controls; p=0.69 LGD compared to controls).

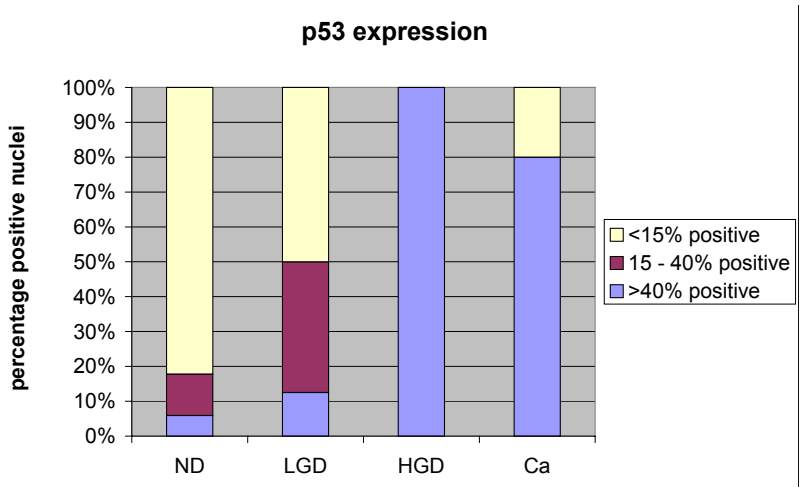

Figure 3: Number of p53 positive nuclei in esophageal biopsies without dysplasia (ND), with low-grade dysplasia (LGD), with high-grade dysplasia (HGD) and with carcinoma.

p53 expression

p53 expression was determined in 133 samples from all 28 achalasia patients (Fig 3). In the control group, 2/48 (4%) biopsy samples (both from one patient) showed p53 overexpression. These biopsies had been taken during the last follow-up endoscopy in this patient. In patients who developed LGD 11/37 (29.7%) biopsy samples were positive (in 67% of patients). In patients esophageal cancer or HGD, 25/48 (52.1%) biopsy samples were positive (in 82% of patients).

The first positive sample had been obtained at a mean of 6 years (range 1- 11 yrs) prior to cancer development (Fig 4). The proportion of biopsy samples with p53 overexpression increased with increasing grades of dysplasia (19/103 (18%) in no dysplasia, 8/16 (50%) in LGD, 3/3 (100%) in HGD and 8/10 (80%) in AC and SSC ($p<0.001$).

Of all 37 p53 positive samples, 18 (49 %) also showed dysplasia or carcinoma.

Correcting for the number of samples per patient and age this indicates a hazard ratio (HR) of 8.0 (95% confidence interval 1.6 - 41) for p53 positive patients to develop carcinoma in comparison to p53 negative patients ($p=0.013$). The HR for patients with LGD to develop carcinoma compared to patients without LGD was 0.98 (95% confidence interval 0.3 - 3.5) ($p=0.97$)

The gradual increase over time in p53 overexpression is shown in Figure 5. From this, it can be seen that p53 overexpression increased at time points closer to the development of esophageal cancer. Moreover, p53 overexpression was already present at a mean of 6 years prior to cancer development at a time when there were no endoscopical signs of progression towards neoplasia.

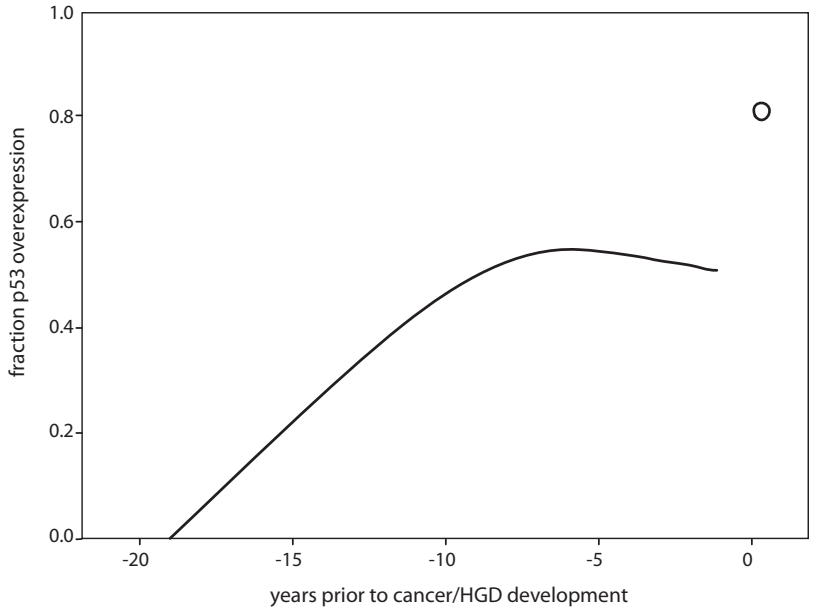

Figure 4: Fraction of patients with a positive p53 sample in the years prior to HGD or cancer development.

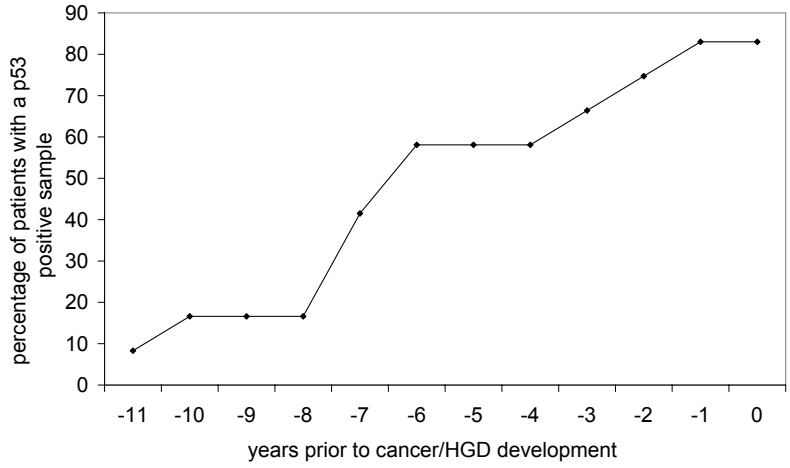

Figure 5: Overexpression of p53 in esophageal biopsy samples from achalasia patients taken some years prior to HGD or cancer development. At t=0 (presence of HGD or esophageal cancer) the fraction of p53 overexpression is 83%.

DISCUSSION

Patients with achalasia have a significantly increased risk of developing of esophageal cancer, both squamous cell carcinoma and adenocarcinoma. This is thought to be related to chronic food stasis and impaired esophageal clearance, which often persists after dilation treatment. In this study we therefore focused on both tumour types. Achalasia patients with esophageal cancer often have an even more dismal prognosis than other esophageal cancer patients. This is at least partly due to the fact that these patients are used to symptoms of impaired food passage and often report worsening of their symptoms at a stage of advanced malignancy [19]. This warrants the consideration for surveillance endoscopies in these patients. However the benefit of surveillance endoscopies in achalasia is disputed because a diagnosis of neoplasia in achalasia at an early and therefore presumably curable stage is often difficult. The findings of this longitudinal analysis show that easily to apply biomarkers such as p53 can be used for the detection of patients at an increased risk of neoplastic progression.

Almost all patients with achalasia, with or without future development of HGD or esophageal cancer, showed overexpression of ki67 in biopsies taken from the esophagus at time points before macroscopically or microscopically presence of neoplastic changes. Consequently, this marker was found to be not discriminative between patients at risk or those not at risk for the development of esophageal cancer in achalasia.

The human ki67 protein is present during all active phases of the cell cycle (G1, S, G2, M), but is absent in resting cells (G0). Although some of its features have been characterized, such as phosphorylation and transport to the cell nucleus for expression to become evident, the exact function of the ki67 protein is still largely unknown. Expression of the ki67 protein is strictly associated with cell proliferation. The fraction of ki67 positive cells has been demonstrated to correlate with the clinical course of the neoplastic disorder [20]. Our results confirm the previously reported presence of hyperproliferation in the esophageal mucosa of achalasia patients [3].

It can be assumed that this hyperproliferation is one of the risk factors for development of LGD, HGD and esophageal cancer in these patients with achalasia. The hyperproliferation is likely caused by the commonly observed food stasis and fermentation in the distal part of the esophagus in these patients. To test this hypothesis, biopsies taken at higher levels in the esophagus should be examined assuming that distally more stasis and therefore, more hyperproliferation is present. In the current study, surveillance biopsies were always taken 1- 2 cm above the GE-junction. Another problem with ki67 expression is the presence of inflammation in esophageal samples of achalasia patients. Inflammation causes to extend ki67 expression to upper layers of the epithelium. This can be another explanation for the high number of ki67 positive samples.

Overexpression of p53 was more frequently observed in achalasia patients who developed esophageal cancer at a later time point. Of the 12 investigated esophageal cancer or HGD

patients, only 5 displayed histological evidence of dysplasia prior to cancer/HGD development, whereas 10 patients already showed p53 overexpression at an earlier stage (Fig 5). Most importantly, this overexpression was already noted at a mean of 6 years prior to cancer development and was a significant predictor for progression towards neoplasia (HR 8.0 (95% confidence interval 1.6 - 41). The hazard ratio was corrected for the fact that the number of samples differed between patients and that the patients in the control group were younger. It is important to stress, that the presence of LGD did not affect this ratio.

The p53 tumour suppressor gene is located on the 17p13 chromosome and is involved in controlling cell proliferation. Normally, cells contain low levels of wild-type p53 which regulates two common responses to oncogenic stress, i.e. cell cycle arrest/DNA repair and apoptosis. In cells that are early in the G1-phase, p53 triggers a checkpoint blocking further progression through the cell cycle, allowing the damaged DNA to be repaired before the cell enters the S-phase [21]. If the DNA damage cannot be repaired, p53 induces apoptosis [22]. This suggests that failure of p53 to respond to DNA damage will increase the susceptibility to oncogenic changes.

Mutated p53 is dominantly negative and it overwhelms the wild-type protein and prevents it from functioning [23]. These p53 mutations are associated with an increased half-life of the p53 protein, resulting in the accumulation in the cell nucleus to levels that can be detected by immunohistochemistry [24]. In contrast, wild-type p53 has a short half-life and, as a consequence, these proteins do not accumulate and are mostly below the threshold of being detected by immunohistochemistry [25]Approximately 90% of p53 mutations are point mutations [26].

Although immunohistochemistry for detecting mutated p53 is cheap, quick and easy to apply compared with other techniques, there are some additional limitations that are important to consider. The p53 antibodies that are commonly used do not only stain the mutant p53 mutation but probably also the wild-type [25]. A second limitation of p53-based immunohistochemistry is that mutations for this tumour suppressor gene may exist without protein overexpression, and therefore will not be detected by immunohistochemistry [25-27]. As with ki67 expression, presence of inflammation can influence p53 evaluation. It is important to stress that only intense red cells were considered p53 positive.

Nevertheless these limitations, our results suggest that p53 is an early marker of neoplastic progression in achalasia.

In the current study, we controlled for duration of disease; however, the three patient groups differed with respect to age, with esophageal cancer patients being the oldest at the time of achalasia development and the control patients being the youngest. The most likely explanation for this is that we selected on outcome (cancer, LGD, no dysplasia), which is related to age. In the reported hazard ratio of 8.0 a correction for age was made.

CONCLUSION

This longitudinal cohort study suggests that p53 but not ki67 can well be used to identify achalasia patients at the highest risk of developing malignancy. Consequently, achalasia patients with p53 overexpression may benefit from a more intensive surveillance interval and possibly the use of newer imaging techniques such as high resolution endoscopy or chrome endoscopy to detect neoplastic changes at an earlier and therefore potentially curable stage. This should however be studied in a prospective follow-up study.

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Chapter 7 Treatment

Predictors for outcome of failure of balloon dilatation in patients with achalasia

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ABSTRACT

Background: Pneumatic balloon dilatation (PD) is a regular treatment modality for achalasia. The reported success rates of PD vary. Recurrent symptoms often require repeated PD or surgery.

Objective: To identify predicting factors for symptom recurrence requiring repeated treatment.

Methods: Between 1974 and 2006, 336 patients were treated with PD and included in this longitudinal cohort study. The median follow-up was 129 months (range 1-378). Recurrence of achalasia was defined as symptom recurrence in combination with increased lower esophageal sphincter (LES) pressure on manometry, requiring repeated treatment. Patient characteristics, results of timed barium esophagram and manometry, as well as baseline PD characteristics were evaluated as predictors for disease recurrence with Kaplan-Meier curves and Cox regression analysis.

Results: 111 patients suffered from symptom recurrence requiring repeated treatment. Symptoms recurred after a mean follow-up of 51 months (range 1-348). High recurrence percentages were found in patients younger than 21 years in whom the 5-year and 10-year risk of recurrence were 64% and 72%, respectively. These risks were respectively 28% and 36% in patients with classic achalasia, respectively 48% and 60% in patients without complete obliteration of the balloon's waist during PD and respectively 25% and 33% in patients with a LES-pressure of > 10 mmHg at 3 months post-dilatation. These four predictors remained statistically significant in a multivariable Cox analysis.

Conclusion: Although PD is an effective primary treatment in patients with primary achalasia, patients are at risk for recurrent disease, with this risk increasing during long-term follow-up. Young age at presentation, classic achalasia, high LES pressure 3 months after PD, and incomplete obliteration of the balloon's waist during PD are the most important predicting factors for the need of repeated treatment during follow-up. Patients who meet one or more of these characteristics may be earlier considered for alternative treatment, such as surgery.

KEYWORDS:

achalasia, pneumatic balloon dilation, predictor, recurrence

STUDY HIGHLIGHTS

1 What is already known about this subject:

Several studies have reported success rates of PD between 35% and 85%.

The previous identified risk factors for symptom recurrence after PD were mainly young age (< 40 years), a post-treatment LES pressure above 10 mmHg and a short period of symptoms prior of treatment.

Laparoscopic myotomy combined with an antireflux procedure provides better symptom relief than all endoscopic and other surgical approaches, with a low complication rate.

2 What are the new findings:

After treatment with PD we found a 5-year risk of recurrence of 28%.

Given the long follow-up period in our study, we were able to show that the rate of recurrence does increase over time.

Young age at presentation, a manometric pattern of classic achalasia, high LES pressure 3 months after PD, and incomplete obliteration of the balloon's waist during PD are the most important predicting factors for the need of repeated treatment during follow-up.

3 How might it impact on clinical practice in the foreseeable future?

This implies that a stricter follow-up can be given to patients with the above mentioned predicting factors, as the chance of recurrence is significantly higher in this subgroup. Using a stricter follow-up, recurrence (defined as symptom recurrence in combination with objective parameters such as a significant increase in LES pressure) can be diagnosed at an earlier stage.

Patients who meet one or more of these characteristics may be earlier considered for alternative treatment, such as surgery.

INTRODUCTION

Achalasia is a rare condition, with an estimated annual incidence of 1 per 100,000, characterized by a functional obstruction of the esophagus caused by failed relaxation of the lower esophageal sphincter (LES) in combination with absent peristalsis of the distal esophagus[1]. The etiology of achalasia is still unknown. The disease is characterized by a loss of inhibitory nitrinergic neurons in the esophageal myenteric plexus. Proposed causes include genetic inheritance, neuronal degeneration, viral infection, and autoimmune disease [2]. The characteristic symptoms of patients with achalasia are dysphagia for solids and liquids, regurgitation of undigested food or saliva, respiratory complaints (nocturnal cough, aspiration), retrosternal pain and weight loss. Therapeutic options aim to improve esophageal emptying by decreasing LES pressure. There are several treatment options, either endoscopic (botulinum

toxin injection and pneumatic balloon dilatation) or surgical[3]. Most gastroenterologists prefer pneumatic balloon dilatation as first line therapy. Nevertheless, the reported success rates of pneumatic dilatation vary widely, ranging in previous studies from 35 to 85 percent within several years of follow-up [4-11]. These marked differences may be partly explained by differences in the length of follow-up. Recurrence of symptoms often requires repeated pneumatic dilatation or further treatment with surgery. The major determinants for pneumatic dilatation therapy failure are insufficiently known: such knowledge would be clinically relevant as it may aid in targeted surveillance and early intervention in high-risk groups. Several studies therefore tried to identify predicting factors of clinical outcome following pneumatic balloon dilatation. Some did not find any specific predictor [10]. Other studies found that advanced age, female gender, a long history of symptoms prior to diagnosis, high pre-therapeutic LES pressure, a post-dilatation LES pressure of < 10 mmHg, limited contrast retention on barium esophagram post dilatation, and a higher number of repeated baseline pneumatic dilatations were associated with a favourable treatment outcome [5, 6, 8, 11-15]. These studies were often performed with relatively small groups of patients. Aim of this study therefore was to identify predicting factors for symptom recurrence requiring repeated pneumatic dilatation in a large cohort of patients with primary achalasia.

PATIENTS AND METHODS

Patients

From 1974 to 2006, 433 patients (214/219 M/F), mean age 51.1 ± 21.6 years (range 4-92) were diagnosed with primary achalasia in our centre. Their data were prospectively included in a database, and they were treated and followed according to a strict patient management, treatment and follow-up protocol, which remained unchanged throughout the study period.

Diagnosis

The diagnosis of achalasia was based on results from upper GI endoscopy, manometry and timed barium esophagram.

Endoscopy

Upper GI endoscopy using a fiber- or video-endoscope (Olympus Europe, Hamburg, Germany) was performed to rule out secondary achalasia.

Esophageal manometry

Esophageal manometry was performed using a low compliance, pneumohydraulic water infusion system (Medical Measure Systems (MMS), Enschede, the Netherlands) and a four-channel silicone catheter (MMS, Enschede, the Netherlands). From May 2005 onwards we used an eight-channel water-perfused manometry assembly with an incorporated 6 cm long

reversed-perfused sleeve sensor (Dentsleeve International Ltd, Mississauga, ON, Canada). The recording sites were connected to an eight-channel polygraph (MMS). The four channel catheter was introduced with all four recording sites in the stomach and then manually pulled back per centimeter with a wet swallow of 5cc water at each centimeter. The eight-channel catheter was introduced in the stomach and then manually pulled back till the sleeve was positioned at the level of the LES. Mean resting LES pressure was calculated as the mean end-expiratory LES pressure during ten wet swallows. LES relaxations were considered to be absent if no change was recorded after swallowing and complete if swallow-induced LES pressure returned to the basic intra-gastric level. A pressure fall above basic gastric level was considered as partial relaxation. Peristaltic wave amplitudes were also calculated as mean wave amplitudes during ten wet swallows. A-peristalsis was diagnosed if all contractions after swallowing were simultaneous. We differentiated two patterns of aperistalsis. Classic achalasia was defined with the presence of non-transmitted or simultaneous contractions with a low amplitude. Vigorous achalasia was diagnosed if there were simultaneous contractions with an amplitude of more than 37 mm Hg.

Timed barium esophagram

Timed barium swallow was performed in standing position after an overnight fast. The patient was asked to drink 200 cc barium solution (or as much as tolerated without regurgitation or aspiration) and recordings of the esophagus were performed at 0, 1 and 10 minutes after the last barium swallow. The appearance of a so-called "bird's beak" was recorded, and the height and maximal width of the barium column were measured at 1 and 10 minutes.

Pneumatic dilatation

From 1974 till 1976, homemade pneumodilatators were used. Since then, dilatation balloons became commercially available, and PD procedures were performed using a Rigiflex balloon (Boston Scientific, Natick, MA, USA).

Baseline pneumatic dilatation was performed on three consecutive days with balloons of either the same or incremental (30, 35, and 40 mm) diameter. We initially used a 40 mm balloon for all three consecutive dilatations. Under the assumption of lower perforation risk, we later adapted this protocol to a graduated approach using balloons with incremental diameters of 30, 35, and 40 mm respectively, inflated like before on successive days. In case of recurrence, we performed a new series of dilatations on 3 consecutive days, again with the same protocol as used for initial treatment. After changing our protocol to the graduated approach, all patients with a recurrence were treated with this protocol, irrespective of the earlier balloon diameter protocol used. Under conscious sedation the balloon was positioned fluoroscopically at the gastro-esophageal junction and inflated to a pressure of 300 mm Hg for 1 minute. Till mid-2001 patients were hospitalized for 4-5 days, but later we switched to an outpatient procedure.

We only performed a post-procedural esophagram using water-soluble contrast on suspicion of a perforation after pneumatic dilatation. Dilatation of the esophagus to any extent on a timed barium esophagram was no exclusion criterion for PD.

Post dilatation follow-up

Following pneumatic dilatation, all patients had a strict schedule of follow-up at 3, 12, 24 months and then every 2 years. Surveillance included upper G-I endoscopy, manometry and timed barium esophagram. After 8 years of follow-up surveillance included upper G-I endoscopy, further examination was performed on indication. This schedule was consistently followed for all patients from 1974.

Recurrence of achalasia was defined as relapse of symptoms (in particular dysphagia, regurgitation and weight loss) in combination with an increased LES pressure on manometry in comparison with the prior LES pressure, requiring further treatment. We did not use a specific quantified symptom instrument to define symptom recurrence. Primary failure of treatment was defined as persistence or recurrence of symptoms in the first 3 months after pneumatic dilatation.

Evaluation of predictors for disease recurrence

Patient characteristics (age at presentation, gender), the results of timed barium esophagram, manometry before treatment, pneumatic dilatation balloon characteristics (stable vs. incremental diameter, and complete vs. incomplete obliteration of the balloon's waist during dilation), as well as the results of repeated manometry 3 months post-treatment were evaluated as predictors for disease recurrence.

Time to recurrence was calculated from first pneumatic dilatation. Kaplan-Meier curves were constructed to estimate the 5 and 10-year cumulative incidence of recurrence. Cox proportional hazards regression was used to estimate univariable and multivariable hazard ratios. Associations between continuous predictors and recurrence were analysed with restricted cubic spline functions on the log hazard scale [16]. These splines allow for a flexible fit the non-linear effects of predictors with relatively few degrees of freedom (df). The predictive strength of each predictor in the multivariable model was indicated by a chi-square statistic, based on the difference in –2 log likelihood of a model with and without the predictor [17]. Statistical analysis was performed using SPSS (version 12.0, SPSS Inc, Chicago, IL, USA), and R software (version 2.5.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients characteristics

From 1974 to 2006, 433 patients were diagnosed with achalasia based on the above-mentioned criteria. Thirty-seven of them did not undergo pneumatic dilatation for various reasons including failure of prior treatment elsewhere, or direct preference for cardiomyotomy. The remaining 396 patients were treated with pneumatic dilatation. Sixty (15.2%) of them were excluded from analysis since they had undergone previous endoscopic or surgical treatment elsewhere. The remaining 336 patients were included for analysis, including 161 men and 175 women, with a mean age at initial presentation of 50 years (range 4 - 92 yrs). In 288 of 336 patients a manometric subclassification of achalasia could be made. There were 222 (77%) patients with classic achalasia and 66 (23%) patients with vigorous achalasia. The median follow-up of all patients was 129 months (range 1-378). During follow up 94 (28%) patients died and 74 (22%) patients were lost to follow up. Two-hundred and twelve patients (63%) were at baseline treated with the fixed 40 mm balloon protocol, 63 (19%) were treated with the incremental balloon protocol, and the remainder 61 patients were treated with another dilatation protocol, fixed 30 mm balloon, fixed 35 mm balloon or unknown protocol.

Complications

Among a total of 985 pneumatic dilatations performed in 336 patients, 40 (4.1%) procedures were associated with one more complications. The complications are summarized in Table 1. In 13 (1.3%) of the 985 pneumatic dilatations, an oesophageal perforation occurred. Two of these patients required surgery; one patient underwent primary repair of the esophagus and one patient esophageal resection. An esophageal stent was temporary placed in one patient. The other 10 patients underwent conservative treatment with antibiotics and no oral feeding. There was no mortality and all patients had a good clinical outcome and were discharged 17±7.9 days after the perforation. Perforation occurred in 5 (2.4%) of the 212 patients who were repeatedly treated with a 40 mm balloon, versus 1 (1.6%) of the 63 patients who were treated with incremental size balloons.

Complication	*Nr of procedures (n=985)*	*Percentage (%)**
None	945	95.9
Post-procedural pain	31	3.1
Fever	16	1.6
Perforation	13	1.3
Aspiration	3	0.3
Bleeding	2	0.2

Table 1 Complications related to PD (* proportions do not add up to 100% as some patients had more than one complication, such as the combination of post-procedural pain, fever, and perforation)

Six perforations occurred in patients who were treated with a fixed dilatation protocol with a 35 mm balloon. One perforation occurred in a patient with an unknown dilatation protocol. Perforations occurred in 5 / 161 (3.1%) males versus 8/ 175 (4.6%) females (p<0.001). They were also more common in patients with complete obliteration of the balloon waist (complete vs. incomplete 5.1 vs. 1.8%, p<0.001). There were no associations between perforation rates and age, LES pressure on manometry before pneumatic dilatation, or duration of achalasia symptoms before diagnosis.

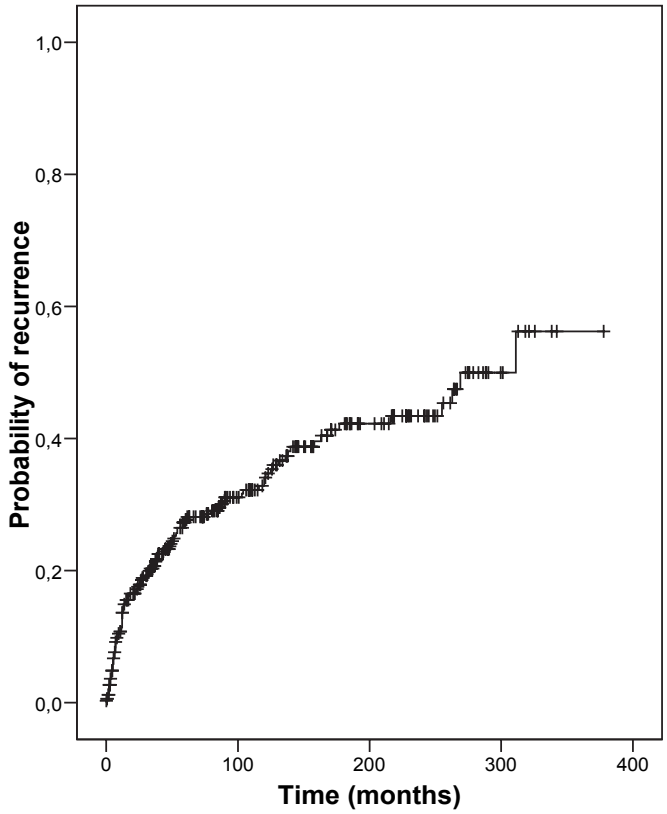

Figure 1 Kaplan-Meier curve for the probability of symptom recurrence after successful primary pneumatic balloon dilatation for achalasia

Overall outcome

During follow-up, 111 of the 336 patients developed recurrence of achalasia requiring further treatment. These first recurrences occurred at a mean interval of 51 months (range 1-348 months) after baseline treatment (Figure 1). In 12 patients the symptoms persisted or recurred within the first 3 months after pneumatic dilatation

Recurrences were treated with renewed pneumatic dilatation in 88 (79%) of the 111 patients, the remainder received other treatment modalities, in particular cardiomyotomy (9%) and botulinum toxin injection (3.6%). The five-year risk of recurrence was 28%; the ten-year risk of recurrence was 34% (Table 2).

Forty-six of the 88 patients who had been treated with repeated pneumatic dilatation for disease recurrence, developed second recurrence of achalasia at a mean interval of 70 months (range 13-372 months) after renewed pneumatic dilatation. Second recurrences were treated with renewed pneumatic dilatation in 34 (74%) of the 46 patients. Fifteen of those 34 patients developed a third recurrence of achalasia at a mean interval of 109 months (range 22-242 months) after renewed pneumatic dilatation and were then treated with renewed pneumatic dilatation in 9 (60%) of the 15 patients.

Predictors of outcome: univariate analysis

The risk of recurrence was significantly higher in younger patients, those with classic achalasia, those with incomplete obliteration of the balloon's waist during pneumatic dilatation, and patients with an LES pressure > 10 mmHg at 3 months after treatment (Table 2). The other factors tested, including gender and balloon diameter used during pneumatic dilatation, did not influence the risk of recurrence. Furthermore, the presence or absence of esophageal dilation on a timed barium esophagram at presentation, as well as LES pressure before treatment did not significantly differ between patients with or without recurrence of achalasia during follow-up.

Table 2 shows that in 10 patients, a presumptive diagnosis of achalasia was made despite low LES pressures on manometry. In view of the clinical picture with typical symptoms, a fully compatible barium esophagram, and a non-relaxing LES with stasis during endoscopy without signs of obstructing lesions, the presumptive diagnosis of achalasia was made and patients were treated with balloon dilatation. The response to this therapy in all 10 patients with improvement of both symptoms and barium esophagram supported the diagnosis of achalasia and suggest that the low LES pressures were due to misreading in the manometry. These patients were therefore included in our analysis, separate analysis with exclusion of these 10 patients did not lead to any major changes in results.

Multivariable analysis

Multivariable analysis showed that age at presentation under 50 years (with increased risk in patients with a younger age), classic achalasia, incomplete obliteration of the balloon's waist during pneumatic dilatation, and LES pressure at 3 months after treatment over 12 mm Hg were significant independent factors to predict the development of later recurrence (all p-values < 0.001). No significant interaction effects were found between these risk factors (all p>0.05), and no simple combinations of characteristics of high risk patients could be found.

Characteristic	*N*	*Recurrence (n)*	*5 yr risk of recurrence (SE)*	*10 yr risk of recurrence (SE)*	*Hazard ratio (95% CI)*	*P-value*
Sex						
Male	161	52	0.27 ± 0.04	0.33 ± 0.04	1.0	0.93
Female	175	59	0.29 ± 0.04	0.37 ± 0.04	1.02 (0.7-1.5)	
Age (years) at onset						
< 21	31	23	0.64 ± 0.9	0.72 ± 0.09	1.0	< 0.001
21-40	91	42	0.31 ± 0.5	0.38 ± 0.06	0.4 (0.3-0.7)	
40-50	46	11	0.17 ± 0.6	0.33 ± 0.10	0.3 (0.1-0.5)	
> 50	168	35	0.21 ± 0.3	0.24 ± 0.04	0.2 (0.1-0.4)	
Type of achalasia						
Classic	222/288	79	0.28 ± 0.03	0.36 ± 0.04	1.8 (1.0-3.2)	0.032
Vigorous	66/288	15	0.18 ± 0.05	0.21 ± 0.06	1.0	
Timed barium esophagram						
Esophagus dilation yes	275/326	87	0.27 ± 0.03	0.33 ± 0.03	1.0	0.36
Esophagus dilation no	51/326	21	0.31 ± 0.07	0.37 ± 0.07	1.3 (0.8-2.0)	
Manometry before treatment						
LES p ≤ 10 mm Hg	10/282	3	0.29 ± 0.18	0.29 ± 0.18	1.0	0.91
LES p > 10 mm Hg	272/282	88	0.25 ± 0.28	0.32 ± 0.03	0.9 (0.3-3.0)	
Obliteration balloon's waist						
Yes	217/327	45	0.16 ± 0.03	0.19 ± 0.03	1.0	<0.001
No	110/327	62	0.48 ± 0.05	0.60 ± 0.05	3.6 (2.4-5.3)	
Balloon diameter						
Consistent (40 mm)	212	70	0.24 ± 0.03	0.32 ± 0.04	1.0	0.63
Incremental (30-35-40mm)	63	16	0.27 ± 0.07	0.37 ± 0.09	0.1 (0.6-1.9)	
Other	61	25	0.39 ± 0.06	0.42 ± 0.07	1.3 (0.8-2.0)	
Manometry 3 months after treatment						
LES p ≤ 10 mm Hg	82/251	15	0.17 ± 0.04	0.19 ± 0.05	1.0	0.03
LES p > 10 mm Hg	169/251	61	0.25 ± 0.03	0.33 ± 0.04	1.9 (1.1-3.3)	
Total	**336**	**111**	**0.28 ± 0.03**	**0.34 ± 0.03**		

Table 2 Distribution of patient and treatment characteristics in relation to recurrence of achalasia. Hazard ratios were calculated in univariate Cox regression analysis.
(LES p = lower esophageal sphincter pressure, CI = confidence interval, SE = standard error).

Relative hazard threshold in LES-pressure and age

We examined the relation between LES pressure measured at 3 months after pneumatic dilatation and the risk of recurrence in further detail (Figure 2). The higher the LES-pressure post dilatation, the greater the relative hazard ratio for recurrence. We found a threshold value of 12 mm Hg. Above this pressure the risk of recurrence increased more or less linearly. Younger age increased the relative hazard ratio for recurrence (Figure 3). We found a threshold value of 50 years, below which the risk of recurrence increased linearly.

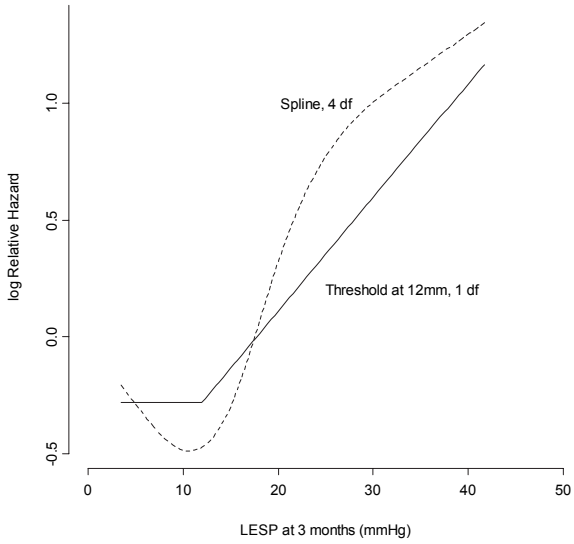

Figure 2 Relative hazard of recurrence of achalasia and LES-pressure 3 months after PD.
The risk of recurrence increases linearly above a threshold value of 12 mm Hg.

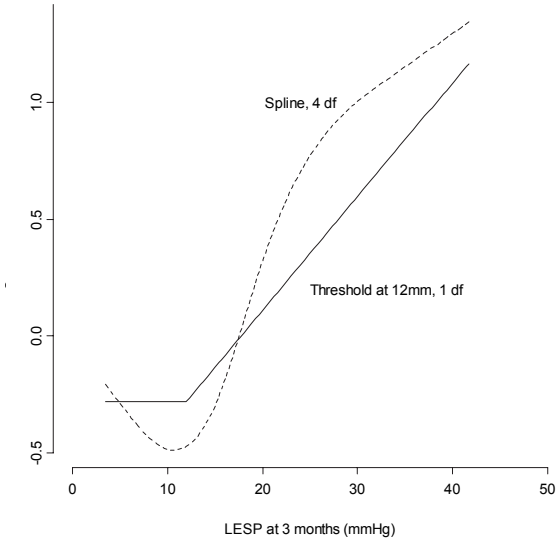

Figure 3 Relative hazard of recurrence of achalasia and age at diagnosis.
The risk of recurrence increases linearly below a treshold value of 50 years.

DISCUSSION

Patients with achalasia are in many countries primarily treated with pneumatic dilation, as this is considered to be a safe and efficient treatment for this condition. Several studies have reported success rates of pneumatic dilatation between 35% and 85% [4-11]. These highly variable results hamper the use of pneumatic dilatation as primary treatment for achalasia.

Variation of success rates is possibly due to different periods of follow-up with a gradual decrease in persistent success rates over time. In a study on 72 patients with a mean follow-up of 6.5 years, the risk of recurrence after pneumatic dilatation remained as low as 15% [10]. Another study showed a persistent treatment success rate of 60% for a patient population followed for 5-9 years post-pneumatic dilatation and 40% for patients followed more than 15 years post-pneumatic dilatation [7]. Our results fit within the above mentioned range of success. However, given the long follow-up period in our study, we were able to show that the rate of recurrence does indeed increase over time.

Another factor that impairs the direct comparison of different study results is the variable definition of recurrence and success. In some studies, recurrence was defined as symptom recurrence, sometimes based on a symptom score questionnaire like the Eckardt score, whereas other studies divided the data on the effectiveness of treatment into four classes according to Vantrappen and Hellemans [7, 18]. This classification is based on symptoms of dysphagia (duration and frequency), regurgitation and weight loss. Others defined success as the absence of the need for further treatment [10]. Against this background, we consider our strict protocol of follow-up after pneumatic dilatation one of the strengths of our study. In case of symptom recurrence we performed an upper GI endoscopy, a timed barium esophagram and a manometry. With this approach, we aimed to confirm an increase in LES-pressures in comparison to previous measurements, thus necessitating repeated treatment.

We deliberately used a clinical definition for persistence and recurrence because this is most applicable and useful in the daily management of achalasia patients. Manometry pressures, timed swallow, or symptoms alone are insufficient tools to diagnose recurrence and repeat pneumatic dilatation or proceed to surgery. Our definition of recurrence was deliberately chosen for optimal clinical management and to prevent misdiagnoses.

Another variable factor in literature concerning pneumatic dilatation is the distension protocol used. Until now there has been no strict, uniformly accepted pneumatic dilatation protocol for achalasia. Some investigators only perform one dilatation [8], others perform several dilatations with incremental diameter of the balloon, or one dilatation at baseline and every couple of weeks after first dilation re-dilatation until clinical remission is achieved [5, 7, 19]. In a retrospective study in a group of 75 achalasia patients, graded dilatation was found to be more effective than a single dilatation [20]. In the present study baseline pneumatic dilatation was performed on 3 consecutive days with balloons of either the same diameter, or incremental (30, 35, and 40 mm) diameter. According to the distension protocol, the majority of

the patients underwent 3 consecutive dilatations with a balloon diameter of 40 mm (n=212) or were treated with balloons with increasing diameter, respectively 30, 35 and eventually 40 mm (n=63). There was no significant difference between both groups with respect to the risk of recurrence.

This study shows that 111 out of 336 patients treated with one series of 3 pneumatic dilatation sessions developed recurrence of achalasia requiring further treatment. These first recurrences occurred at a mean interval of 51 months after baseline treatment. The 5-year risk of recurrence is 28%. In one study with a variable dilatation protocol consisting of one to three initial pneumatic dilatation sessions, a 5-9 year success of 60% was found after treatment with pneumatic dilatation [7]. In another study, in which the investigators performed one baseline pneumatic dilatation followed by on-demand strategy every 2-3 weeks till clinical remission was achieved, a 5-year persistent success of 67% was reported [5]. These studies are in contrast with a study from German investigators which reported no more than 40 % persistent remission five years after one baseline pneumatic dilatation [8]. In comparison with these studies, our 5-year risk of recurrence is relatively low.

After pneumatic dilatation, all patients in the present study had a strict schedule of follow-up at 3, 12, 24 months and then every 2 years. Surveillance included upper GI-endoscopy, manometry and timed barium esophagram. After 8 years of follow-up surveillance included upper GI-endoscopy, further examination was performed on indication. In another study, patients were not routinely followed up after pneumatic dilatation, but were advised to make an appointment when symptoms recurred [7]. We believe that symptoms alone are not reliable in assessing treatment success, but objective assessment of treatment and symptoms are required to achieve a higher treatment success. Patients are used to have a certain level of discomfort due to achalasia. Therefore this group of patients will not present themselves in an early phase of recurrence of disease.

In several studies risk factors for symptom recurrence after pneumatic dilatation have been identified. These factors were mainly young age (< 40 years), a post-treatment LES pressure above 10 mm Hg and a short period of symptoms prior of treatment [5, 6, 8, 12]. A severely dilated esophagus (>80 mm) has been reported to be associated with poor outcome [4, 21].

We found age to be a predictive factor in treatment outcome. Patients younger than 50 years responded less well to pneumatic dilatation. The risk of recurrence increased linearly in patients with an age below 50 years. High recurrence percentages were found in patients younger than 21 years as the 5-year and 10-year risk of recurrence were 64% and 72%, respectively.

In one study achalasia was categorized into 3 subtypes; type I as classic achalasia, type II as achalasia with esophageal compression, and type III as achalasia with spastic contractions. This study analysed treatment outcome in 83 patients and showed each subtype to be distinct in their responsiveness to therapy. Type III was a predictor of negative treatment response [22]. The current study differentiated achalasia in 2 subtypes, classic achalasia (type

I) and vigorous achalasia (type II). This differentiation could be made in 288 patients. We showed that classic achalasia was associated with a higher risk of recurrence.

In several studies treatment response was associated with low LES pressures post-dilatation. The most optimal response was found in patients with a post-dilation LES pressure lower than 10 mm Hg [8, 11, 14]. The current study confirms that a low LES pressure post-dilatation is associated with a good clinical outcome and shows that a LES pressure of < 12 mmHg at 3 months post-dilatation is associated with a significant lower risk of recurrence. We therefore recommend the performance of a manometry at 3 months post-dilatation to measure LES pressure as a predictor of treatment outcome.

Incomplete obliteration of the balloon's waist was found to be a significant predictor of clinical outcome as this subgroup showed a significant higher risk for the development of recurrence. In comparison with patients with complete obliteration, patients without showed a hazard ratio of 3.6.

In the present study the presence of dilation of the esophagus was not associated with increased risk of recurrence; however, we only scored the parameter dilation/no dilation but did not grade the severity of esophageal dilation. This does not exclude the possibility that the level of severity of dilation could correlate with the risk of recurrence.

In a study from the US, investigators showed that patients with a longer duration of symptoms before diagnosis had a more tortuous esophageal body and tended to have an increased esophageal diameter observed by radiography [23]. It can be hypothesized that early detection of recurrent disease and repeated treatment in an early stage may prevent (further) esophageal decompensation. Further investigation needs to be performed to confirm this hypothesis.

There are a few shortcomings to our study. Firstly, we did not correlate improved emptying on a barium esophagram independently with symptom relief, LES pressures, and relapse rates. Secondly, our pneumatic dilatations stopped at a pressure of 300 mmHg. We did not increase the balloon pressure till complete obliteration of the balloon waist was achieved in all patients. Therefore our results are not the ultimate standard that can be achieved by pneumatic dilatation treatment. Further studies have to show whether differentiation of the approach, for example higher pressure, can improve results without increasing complication rates.

Several studies have compared surgery and pneumatic dilatation for the treatment of patients with achalasia [19, 24-27].

In one of these studies 30.3% of patients initially treated with surgical myotomy had another intervention after 5 years and 37.5% of patients had a subsequent intervention 10 years after initial therapy. In our study we obtained similar results with pneumatic dilatation, with a 5 and 10-year recurrence rate of 28% and 34% respectively.

A recently published systemic review showed that laparoscopic myotomy combined with an antireflux procedure provided better symptom relief (90%) than all endoscopic and other surgical approaches, with a low complication rate (6.3%) [28].

Recently, a large European prospective randomised study compared laparoscopic Heller myotomy versus pneumatic dilatation as baseline treatment in 200 achalasia patients [29]. One hundred and six patients were treated with laparoscopic Heller myotomy with Dor fundoplication and 94 patients were treated with pneumatic dilatation. After two years of follow-up, both treatments had a comparable success rate of 92 versus 87%. Further follow-up is required to evaluate long-term outcome. The long-term outcome of this study should further determine which therapy should be reserved for different subgroups of achalasia patients as baseline therapy.

CONCLUSION

Pneumatic dilatation is an effective treatment for achalasia, but recurrence rates after pneumatic dilatation increase over time. Young age at presentation, classic achalasia, incomplete obliteration of the balloon's waist during dilatation, and high LES pressure at 3 months after pneumatic dilatation are independent predicting factors for the need of repeated pneumatic dilatation during follow-up. These factors should be taken into account in the follow-up strategy of achalasia patients, leading to a stricter follow-up protocol in patients with the above-mentioned predicting factors. Such a protocol should aim for timely detection of recurrence (defined as symptom recurrence in combination with objective parameters such as a significant increase in LES pressure) with renewed, tailored treatment. Our results show that most patients are then eligible for renewed pneumatic dilatation. However, selected cases such as those 21 years of age or younger may in case of recurrence be referred for surgery earlier.

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Chapter 8 **Etiology**

Auto-immune thyroid diseases are more common in patients with achalasia

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Submitted

ABSTRACT

Introduction: The etiology of achalasia is still unknown but there is some evidence for a genetic or auto-immune pathofysiology. In this case one should expect familiar clustering of achalasia and an association between achalasia and other auto-immune diseases.

Methods*:* In a large cohort of achalasia patients with long term follow-up we studied the familiar prevalence of achalasia and the prevalence of auto-immune thyroid diseases by means of a questionnaire send to the patient and their general practitioner. Patients older than 75 years were excluded.

Results: Out of the 239 patients with achalasia still under surveillance, 158 were suitable for including in this study. One-hundred-fifteen (73%, 60 male, mean age 52 years range 18-74) patients returned the questionnaire. All general practitioners returned the questionnaire. Nine (7.8%) patients suffered from thyroid disease. Compared to the age and gender identical overall population the odds ratio for the presence of autoimmune thyroid disease in achalasia patients was 6.58 (3.42 – 12.66) ($p< 0.001$). Also other auto-immune diseases were reported but more scarcely.

Four (3.5%) patients reported a first degree relative and 2 (1.7%) patients a second degree relative with symptoms compatible with achalasia but none of these relatives had been diagnosed with or treated for achalasia.

Conclusion: This study in a large group of achalasia patients does confirm the hypothesis that achalasia patients are more prone to auto-immune thyroid disease but does not suggest a familial clustering of the disease.

INTRODUCTION

Achalasia is a motor disease of the esophagus in which aperistalsis and incomplete or absent LES relaxation leads to a functional esophageal obstruction. The etiology of this disease is still largely unknown and therefore treatment is purely symptomatic and aims at reducing the LES pressure by pneumatic dilatation or laparoscopic myotomy[1]. The impaired esophageal motility with resulting chronic stasis as well as the dilation therapy with resulting gastro-esophageal reflux give rise to mucosal inflammation[2], which increases the risk for esophageal cancer[3]

Esophageal resection specimen of patients with achalasia have revealed inflammation around the myenteric plexus [4]. It is also known that the neurons containing nitric oxide are absent in the myenteric plexus in patients with achalasia. Nitric oxide is implicated in the inhibitory response of the lower esophageal sphincter[5]. The inflammatory infiltrate within the myenteric plexus predominantly contains T-lymfocytes (CD3 positive) [4, 6]. This has led to the hypothesis that an autoimmune process may underlie achalasia leading to an inflammatory process and neuronal cell loss. HSV-1 viral antigen have been proposed as evoking agent but due to conflicting evidence the exact etiology remains to be determined [7-9].

The observation that achalasia can occur at every age but shows two incidence peaks at 30 – 40 years and after 60 years of age supports the auto-immune hypothesis [10]. The similar prevalence of achalasia in men and women may be an argument against.

If the disease has an autoimmune etiology, one should expect that other auto-immune diseases, of which auto-immune thyroid disease is the most common, appear more often in patients with achalasia compared to the overall population.

There is also some evidence for a genetic basis for achalasia [11]. Some case-control series have described associations with certain polymorphisms within the *IL23R* and *NOS2A* gene [12], although the latter could not be confirmed in a large series. [13]

For this reasons, we studied the prevalence of other auto-immune diseases, especially auto-immune thyroid disease and the prevalence of achalasia in first and second degree relatives in a large cohort of achalasia patients.

MATERIAL AND METHODS:

Patients

This is a single centre, cross sectional clinical study performed in a university medical centre which since long serves as a tertiary referral centre for treatment and surveillance of patients with achalasia. Our clinical protocol has been described before[1]. In brief, the diagnosis was based on results from upper gastro-intestinal endoscopy, esophageal manometry and timed barium esophagram. All patients were offered treatment with pneumatic dilatation and kept

under follow-up. The first years of follow up consisted of regular endoscopy, manometry and timed barium esophagram. After 7 years only surveillance endoscopy was performed.

For this study, patients under 75 years of age still under endoscopic surveillance were eligible for inclusion. These patients were asked to complete a self-developed questionnaire which focused, amongst others, on co-morbidity and familiar occurrence of achalasia. Informed consent was asked to contact the general practitioner. Patients, who did not return the questionnaire within 8 weeks, received a reminder. A short questionnaire was sent to the general practitioner with the questions whether the patient suffered from thyroid or other auto-immune diseases and if so, when this diagnosis was made. When one of the completed questionnaires raised additional questions the patient or treating doctor was called.

The prevalence of thyroid disease was compared to the age and sex adjusted overall population as reported in a Dutch national survey on the prevalence and incidence in general practice[14].

***Statistics**:*

The prevalence of thyroid auto-immune disease was compared with the prevalence reported in general practice. A comparison of age en sex matched prevalence of thyroid disease between achalasia patients and this control group was made in SPSS, using log linear regression. The odds ratio was calculated based on a model distinguishing patients and the general population, corrected for age and gender.

RESULTS:

Of the 239 patients currently under surveillance, 81 patients were excluded for reasons of age. The remaining 158 (67%) patients were eligible for inclusion and asked to complete the questionnaire. Seventy-three percent of the eligible patients returned the questionnaire (115/159, M/F 60/55). The mean age of the participants was 52 years (range 18-74). The mean duration of disease was 19 years (2-61). Mean age at diagnosis had been 33 years (range 3-67).

The mean age and gender of the 44 non-responders (M/F 21/23, mean age 50 (20-73) years) did not differ from the responders.

Seven (6%) patients reported to suffer from thyroid disease (5 hypo- and 2 hyperthyroidism). The general practitioners confirmed all diagnosis. This survey for confirmatory general practice data revealed two additional patients with hypothyroidism which brought the total prevalence of thyroid disease in our cohort of achalasia patients to 7.8% (9/115 patients). The age- and sex-matched prevalence of thyroid disease in Dutch general practice was 0.9%. As such the odds ratio for the association between achalasia and thyroid disease was 6.58 (Confidence interval 3.42 – 12.66) ($p < 0{,}001$). This indicates that the prevalence of auto-immune

thyroid disease is more than six times higher in a cohort of achalasia patients compared to the overall population.

In five patients the thyroid disease was diagnosed before achalasia was. In the other 4 patients the thyroid disease developed later during follow-up. (Table 1)

Two patients (1.7%) reported Addison's disease, one of them suffering from the Allgrove

Patient	sex	Thyroid disease	Age achalasia diagnosis	Age thyroid disease diagnosis
1	Female	hypothyroidism	18	37
2	Female	hypothyroidism	41	38
3	Female	hyperthyroidism	67	64
4	Female	hypothyroidism	56	54
5	Female	hypothyroidism	64	<64
6	Female	hyperthyroidism	58	63
7	Female	hypothyroidism	39	37
8	Female	hypothyroidism	27	49
9	female	hypothyroidism	53	56

Table 1: age at diagnosis of achalasia and thyroid disease

syndrome or Triple A syndrome consisting of the combination of achalasia, Addison and alacrimia, a rare autosomal recessive congenital disorder. Three patients (2.6%) suffered from rheumatoid arthritis and 2 (1.7%) patients from Crohn's disease both in combination with Bechterew's spondylarthropathy. One patient was known with idiopathic trombocytopenic purpura (ITP). (Table 2) Four patients (3.5%) reported to have diabetes, one insuline dependent.

Six (5.2%) patients reported a first- or second degree (n=4, resp 2) relative with symptoms compatible with achalasia but none of them had actually been diagnosed with achalasia.

The cohort included three twins. None of these sibs had symptoms of achalasia.

The latest reported incidence of achalasia in a Western country is 1.63 per 100.000 and prevalence of 10.82 per 100.000.[15] Therefore our cohort is too small to study familial preponderance.

	Total patients n (%)
Thyroid	9 (7.8%) (OR 6.58) (CI 3.42 – 12.66) P<0.001
Hyperthyreoidism	2
Hypothyreoidism	7
Addison's disease	2 (1.7%)
Allgrove syndrome	1
Reumatoid arthritis	3 (2.6%)
Crohn's disease	2 (1.7%)
M Bechterew	2 (1.7%)
ITP	1 (0.87%)

Table 2: prevalence of auto-immune diseases

DISCUSSION:

One hypothesis for the etiology of achalasia assumes that a viral infection elicits an auto-immune response in an genetically susceptible patient [16]. However this hypothesis has so far never been proven, not in general nor for specific viral infections such as herpes simplex and human papilloma viruses [9].

The literature regarding potential associations between achalasia and other auto-immune diseases is also sparse. Several case reports reported co-occurrence of achalasia and auto-inflammatory or –immune conditions such as auto-immune thyreotoxicosis [17], myasthenia gravis and polymyositis [18, 19]. In two cohorts of 30 respectively 44 achalasia patients, the prevalence of achalasia ranged between 20% and 23% [20, 21]. Recently a paper from Canada, investigating auto-immune conditions in 193 patients with achalasia reported an odds ratio of 8.5 for hypothyreoidism. This study was based on retrospective chart review. Comparison was made with the overall population but the achalasia patients were 10-15 years older than the controls and the achalasia cohort contained more men [22]. We used the age and gender specific prevalence of the general practice and compared these with the data delivered by the general practitioners of the achalasia patients. Therefore our odds ratio is lower (6.58 versus 8.5) but likely to be more realistic.

In our cohort of patients with achalasia the prevalence of auto-immune thyroid disease was more than 6-times larger than in the overall population, corrected for age and sex. This finding supports the hypothesis that achalasia has an auto-inflammatory component. The observation of a variety of other auto-immune diseases in 15 (10%) of our patients is of interest and further supports the link with an auto-inflammatory mechanism.

This underlies the importance for further research into the underlying mechanism. If the auto-immune etiology is strengthened, this might have consequences for the treatment of achalasia. Treatment nowadays is purely mechanistic consisting of LES-pressure lowering therapy by means of pneumatic dilatation or laparoscopic myotomy, which focuses on managing the symptoms instead of the cause[1]. One can argue that immunosuppressive therapy should be considered in early achalasia[23]. If the inflammation at the level of the myenteric plexus can be suppressed, esophageal motility might restore. However patients with achalasia usually present in the later phase of the disease when the plexus is severely damaged or even destroyed and it is unlikely that immunosuppressive therapy can help at that stage.

Given this high prevalence of thyroid disease, it is reasonable to screen achalasia patients for thyroid diseases and be attentive of symptoms of other auto-inflammatory diseases. A comparison can be made with celiac disease in which the prevalence of thyroid disease is around 10% and regular TSH screening is widely accepted[24].

Achalasia in relatives other than in the Allgrove syndrome is very scarcely reported and probably the older publications of familial achalasia [25-35] indeed includes patients with Allgrove syndrome as this syndrome was first described in 1978[36]. In our cohort no first- or

second-degree relatives had actually been diagnosed with or treated for achalasia even though several were reported to have symptoms compatible with achalasia. It will be of interest to perform a further survey in the near future to see whether part of these subjects will have been diagnosed with achalasia by then.

CONCLUSION

Patients with achalasia are more likely to suffer from auto-immune thyroid disease than matched controls. This supports the hypothesis that achalasia has an underlying auto-inflammatory component. We could not confirm a familiar clustering of achalasia.

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Chapter 9

Summary and general discussion

Achalasia is a rather rare motility disorder of the esophagus characterized by a non-relaxing lower esophageal sphincter in combination with esophageal aperistalsis leading to chronic food stasis. Without treatment this stasis causes esophageal dilatation and elongation and eventually can lead to a decompensated esophagus. Fermentation of retained food affects the esophageal mucosa causing inflammation. This inflammation leads to proliferation and can cause multifocal dysplasia and in some patients leads to the development of squamous cell carcinoma.

Treatment of achalasia is purely symptomatic and aims at lowering of the pressure in the lower esophageal sphincter. Adequate treatment may restore food passage, yet simultaneously caries a risk of inducing gastro-esophageal reflux, which can induce development of Barrett's epithelium and eventually esophageal adenocarcinoma. These aspects are further discussed and outlined in **chapter 2**.

In **chapter 3** esophageal biopsy samples obtained during endoscopic surveillance after pneumatic dilatation were studied for the presence of inflammation. The histological findings were compared to the endoscopical report. Forty percent of the achalasia patients developed histological chronic active or ulcerating esophagitis after LES-lowering treatment. The sensitivity of endoscopy to detect inflammation was poor, partly explained by impaired visibility due to mucosal adherence of food. There was a clear association between histological inflammation and food stasis seen at endoscopy. Since the sensitivity of endoscopy to detect inflammation is low, surveillance endoscopy with biopsy sampling and assessment of stasis is warranted to detect inflammation and early neoplastic changes. Efforts should be made to optimize the conditions for endoscopy by prescription of a liquid diet for 2-3 days before surveillance endoscopy to obtain a better view. One can argue that severe inflammation or food stasis at surveillance endoscopy are arguments for a more aggressive treatment protocol even if the patient denies deterioration of symptoms.

In **chapter 4** the risk of esophageal cancer development in longstanding achalasia treated with pneumatic dilatation was studied. In a cohort of 448 patients with a mean follow-up of 9.6 years, 15 (3.3%) patients developed esophageal carcinoma. The hazard ratio to develop esophageal cancer was 28 (CI 17-46). The risk to develop esophageal cancer rose with the length of follow-up and the duration of symptoms. After a follow up of 20 years, the proportion of patients developing an esophageal carcinoma had risen to approximately 10%. This explains our expectation that the hazard ratio for development of esophageal cancer in the presence of achalasia will continue to increase with further follow-up. The risk of cancer development is similar to the risk of cancer development in Barrett's metaplasia for which surveillance is widely accepted. Therefore surveillance in longstanding achalasia should be considered, although the optimal surveillance strategy needs to be defined. In our cohort only 5 (33%) patients were detected at a curable state. Optimization of surveillance might be facilitated by the use of histological markers. For that purpose, we studied in **chapter 5** the use of markers in surveillance biopsy samples. Surveillance biopsies of patients who

eventually developed esophageal carcinoma were studied for p53 and ki67 expression using immunohistochemistry. These were compared with the surveillance biopsy samples from 10 patients with achalasia without cancer development during similar length of observation. Ki67, a proliferation marker was positive in a large amount of samples and did not differ between cancer patients and controls. This is likely a result of hyperproliferation caused by food stasis. P53 however was positive in 82% of the patients at a mean of 6 years before cancer development compared with 10% in the control patients. We therefore concluded that P53 may be used to identify achalasia patients with the highest risk of developing malignancy.

Chapter 6 focused on the risk of development of Barrett's metaplasia. Although it seems reasonable to consider achalasia and Barrett's metaplasia as two opposing ends of a spectrum we actually showed that there is an association between these two conditions. Treatment of achalasia leading to a low LES-pressure may induce gastro-esophageal reflux leading to Barrett's esophagus and esophageal adenocarcinoma. Barrett's metaplasia in achalasia is however scarcely reported, probably because surveillance endoscopy is rarely performed. Barrett's esophagus is also incidentally found in untreated patients, in which case achalasia was likely to develop independent from, yet after, the development of Barrett's metaplasia. In our cohort of 331 patients with achalasia treated with pneumatic dilatation 8.4% of patients developed Barrett's metaplasia. It was remarkable that patients with a hiatal hernia carried the highest risk for Barrett's development. The development of Barrett's metaplasia forms an extra argument to perform surveillance endoscopies. The patients with Barrett's metaplasia or a hiatal hernia should be offered a more intensive surveillance protocol.

Chapter 7 focused on pneumatic dilatation. Pneumatic dilatation is a safe and effective therapy strategy. In a period of 32 years, 336 patients were treated primarily with pneumatic dilatation with increasing balloon size (30, 35 and 40 mm) on 3 consecutive days. The perforation risk was 1.3% without mortality. The 5-years risk of recurrence was 28%, with the first recurrence occurring after a mean interval of 51 months. As follow-up lengthens the recurrence rate increases. Age is a predictive factor in treatment outcome. Patients younger than 50 years responded less to pneumatic dilatation. In patients younger than 21 years the 5-year and 10-year risk of recurrence was even 64% and 72% respectively. Other predictive factors are classic achalasia which was associated with a higher recurrence risk as are LES pressure three months after pneumatic dilatation higher than 12 mmHg and incomplete obliteration of the balloon waist.

Chapter 8 reports on the co-incidence of achalasia and auto-immune thyroid disease. Since the etiology of achalasia is still unresolved, treatment is purely symptomatic aiming at reducing the LES-pressure to improve passage of food. However, there is some evidence that achalasia is an autoimmune disease. If this is true, a coincidence with other auto-immune diseases is expected. The prevalence of autoimmune thyroid disease was studied and compared to the prevalence in the overall population. In our cohort indeed a higher prevalence of auto-immune thyroid disease was found (OR 6.58 (3.42 – 12.66)). Also other auto-immune

diseases were reported. This finding supports the auto-immune hypothesis. A familiar occurrence, which is also common in auto-immune diseases, could not be detected. This is probably due to the cohort size, which is too small to study a familiar clustering of a disease with a very low prevalence.

In conclusion achalasia is a rare motility disorder with evidence for an autoimmune etiology as autoimmune thyroid diseases appear more common in patients with achalasia. However this observation has not lead to causative treatment. Treatment is still symptomatic at lowering the lower esophageal sphincter pressure.

Pneumatic dilatation is an effective LES pressure lowering treatment module, however young age, classic achalasia, high LES-pressure 3 month after PD and incomplete obliteration of the balloon's waist are important predictors for the need of repeated treatment and alternative treatment as surgery should in these cases be considered.

Patients with achalasia have a considerable risk to develop esophageal carcinoma, which is often detected in a late incurable state. Efforts should be made to define those patients with the highest risk, who could benefit from a more intense surveillance protocol. Long lasting disease, p53 overexpression and inflammation in esophageal surveillance biopsy samples, food stasis at endoscopy and development of Barrett's metaplasia appeared to be independent risk factors. Future research should focus on the best surveillance interval and strategy. The patients with risk factors should be offered a, probably annual, surveillance endoscopy starting 10 years after onset of symptoms. To improve the yield of a surveillance endoscopy the esophagus should be properly cleaned by prescribing the patients with food stasis a liquid diet 2-3 days before endoscopy.

In case of food stasis at endoscopy or severe inflammation in the surveillance biopsy samples retreatment should be considered even in the absence of deterioration of symptoms as these are important risk factors.

To prove this strategy, a prospective randomized study is needed but will be hard to perform. Therefore it is important to cluster and follow achalasia patients by a strict protocol, to study the cancer risk as follow-up lengthens and to study the outcome of a more intensive surveillance and treatment protocol.

Chapter **10**

Samenvatting en discussie

Achalasie is een vrij zeldzame aandoening van de slokdarm, welke wordt gekarakteriseerd door een niet of onvoldoende relaxerende onderste slokdarmsphincter in combinatie met afwezige slokdarmperistaltiek. Tezamen leidt dit tot klachten van een functionele slokdarmobstructie met chronische stase van voedsel. Zonder behandeling kan deze voedselstase leiden tot dilatatie en elongatie van de slokdarm en uiteindelijk zelfs tot een gedecompenseerde slokdarm. Fermentatie van geretineerd voedsel heeft een etsend effect op de slokdarm mucosa en veroorzaakt ontsteking. Deze chronische ontsteking veroorzaakt proliferatie en kan uiteindelijk leiden tot multi-focale dysplasie en in sommige patiënten zelfs tot de ontwikkeling van een plaveiselcelcarcinoom van de slokdarm.

De behandeling van achalasie is puur symptomatisch en gericht op het verlagen van de druk in de onderste slokdarmsphincter. Adequate behandeling kan de voedselpassage verbeteren maar zal tegelijkertijd een risico inhouden op het ontstaan van gastro-oesofageale zure reflux, welke het ontstaan van Barrett's metaplasie en uiteindelijk zelfs de ontwikkeling van een adenocarcinoom kan induceren. Deze aspecten werden verder uitgewerkt en bediscussieerd in **hoofdstuk 2**.

In **hoofdstuk 3** werden slokdarmbiopten, die zijn verkregen in het kader van surveillance na pneumodilatatie, opnieuw beoordeeld op de aanwezigheid van ontsteking. Deze histologische bevindingen werden vergeleken met de bevindingen gerapporteerd in het endoscopie verslag. Veertig procent van de patiënten met achalasie ontwikkelde chronisch actieve of ulcererende oesofagitis in de biopten na hun behandeling voor achalasie. De sensitiviteit van endoscopisch onderzoek om ontsteking vast te stellen bleek slecht, deels verklaard door bemoeilijkte endoscopische inspectie als gevolg van voedselstase en voedselbeslag op de mucosa. Er blijkt een duidelijke associatie aanwezig tussen de histologische ontsteking en de voedselstase vastgesteld bij endoscopisch onderzoek.

Aangezien de sensitiviteit van endoscopisch onderzoek om ontsteking te detecteren laag is, is surveillance endoscopie met het nemen van biopten en het objectiveren van voedselstase aangewezen om ontsteking en vroege neoplastische veranderingen op te sporen. Maatregelen zoals het voorschrijven van een vloeibaar dieet 2-3 dagen voor endoscopisch onderzoek zijn nodig om de inspectie van de mucosa te optimaliseren. Het is te overwegen om in het geval van ernstige ontsteking in de biopten of voedselstase bij surveillance endoscopie een agressievere houding aan te nemen en aanvullende behandeling te overwegen zelfs indien de patiënt geen duidelijke toename van zijn klachten aangeeft.

In **hoofdstuk 4** werd het risico op het ontwikkelen van een slokdarmcarcinoom voor patienten met lang bestaande achalasie na pneumodilatatie bestudeerd. In ons cohort van 448 patiënten met een gemiddelde follow-up van 9,6 jaar ontwikkelden 15 (3,3%) patiënten een slokdarmcarcinoom. De hazard ratio om een slokdarmcarcinoom te ontwikkelen bedraagt 28 (95% betrouwbaarheidsinterval 17-46). Het risico om een slokdarmcarcinoom te ontwikkelen neemt toe met de lengte van de follow-up en de duur van de klachten. Na een follow-up van 20 jaar loopt het percentage patiënten, dat een slokdarmcarcinoom ontwikkelt, zelfs op tot

ongeveer 10%. Het is te verwachten dat de hazard ratio verder zal oplopen als de duur van follow-up langer wordt.

Het risico op het ontwikkelen van een slokdarmcarcinoom voor patiënten met achalasie is vergelijkbaar met het ontwikkelen van een carcinoom bij patiënten met een Barrett's metaplasie, waarvoor surveillance algemeen is geaccepteerd. Daarom moet surveillance bij patiënten met langdurige achalasie sterk overwogen worden, hoewel de optimale surveillance strategie nog zeer onduidelijk is. In ons cohort werden namelijk ondanks een 3-jaarlijkse surveillance slechts 5 (33%) patiënten gediagnosticeerd in een resectabel stadium. Voor het optimaliseren van surveillance zouden wellicht histologische markers gebruikt kunnen worden. Met deze vraagstelling werd in **hoofdstuk 5** het gebruik van markers op surveillance biopten verder bestudeerd. Surveillance biopten van patiënten die uiteindelijk een slokdarmcarcinoom ontwikkelden werden onderzocht op expressie van p53 en ki67 met behulp van immunohistochemisch onderzoek. Deze gegevens werden vergeleken met surveillance biopten van 10 patiënten met achalasia die gedurende een vergelijkbare follow up lengte geen carcinoom ontwikkelden.

Ki67 is een proliferatiemarker en bleek positief in een groot aantal biopten zonder verschil tussen patiënten die kanker ontwikkelden en de controle groep. Dit is waarschijnlijk het gevolg van hyperproliferatie veroorzaakt door chronische voedselstase en ontsteking. P53 echter, bleek positief in 82 % van de patiënten die uiteindelijk kanker ontwikkelden vergeleken met 10% in de controle groep. We concludeerden dat p53 gebruikt kan worden om de achalasia patiënten met het hoogste risico op het ontwikkelen van een maligniteit te identificeren.

Hoofdstuk 6 focust op het risico op het ontwikkelen van Barrett's metaplasie. Hoewel het logisch lijkt om achalasie en Barrett's oesofagus als twee tegenovergestelde uiteinden van een spectrum te zien hebben we aangetoond dat er een associatie bestaat tussen beide aandoeningen. Behandeling van achalasie gericht op het verlagen van de druk in de onderste slokdarmsphincter kan gastro-oesophageale reflux veroorzaken, leidend tot Barrett's metaplasie en adenocarcinoom van de slokdarm. Barrett's metaplasie bij patiënten met achalasie is slechts schaars beschreven, waarschijnlijk omdat surveillance bij achalasia weinig wordt verricht. Verrassenderwijs is Barrett's metaplasie ook enkele malen gerapporteerd bij patiënten met achalasie die nog niet behandeld zijn. Achalasie lijkt zich dan onafhankelijk van of na het ontstaan van de Barrett te ontwikkelen. In ons cohort van 331 patiënten met achalasie, die hoofdzakelijk met pneumodilatatie zijn behandeld ontwikkelde 8,4% van de patiënten Barrett's metaplasie. Het was opvallend dat de patiënten met een hiatus hernia het hoogste risico hadden op het ontwikkelen van Barrett's metaplasie. De ontwikkeling van een Barrett's slokdarm is een extra argument om surveillance uit te voeren bij patiënten met achalasie. De patiënten met Barrett of een hiatus hernia moeten mogelijk een intensievere surveillance aangeboden krijgen.

Hoofdstuk 7 onderzoekt pneumodilatatie. Pneumodilatatie is een veilige en effectieve behandelmethode. Gedurende een periode van 32 jaar werden 336 patiënten met achalasie in ons centrum primair behandeld met pneumodilatatie met opklimmende ballondiameter (30, 35 en 40 mm) op 3 opeenvolgende dagen. Het perforatierisico bleek laag 1,3% en zonder mortaliteit. Het 5-jaars risico op recidief klachten was 28% met het eerste recidief gemiddeld 51 maanden na de behandeling. Bij oplopen van de follow-up duur neemt dit recidief risico toe. Leeftijd is een voorspellende factor voor de uitkomst van de behandeling. Patiënten jonger dan 50 jaar hebben minder (lang) effect van pneumodilatatie. Patiënten jonger dan 21 jaar hebben zelfs een recidief risico van 64% en 72%, na 5 respectievelijk 10 jaar. Andere voorspellende factoren zijn klassieke achalasie, een rustdruk in de onderste sphincter van > 12 mm Hg 3 maanden na behandeling en incompleet verstrijken van de taille in de ballon tijdens dilatatie. Deze factoren gingen alle gepaard met een hoger risico op recidief.

Hoofdstuk 8 rapporteert de co-incidentie van achalasie en auto-immuun schildklierziekten. Omdat de etiologie van achalasie nog niet is opgehelderd is de behandeling puur symptomatisch en gericht op het verlagen van de rustdruk in de onderste slokdarmsphincter en verbeteren van de voedselpassage. Echter, er is enig bewijs dat achalasie een auto-immuun aandoening betreft. Als dit het geval is, dan zou er een hogere prevalentie van andere auto-immuun aandoeningen bestaan in de groep patiënten met achalasie. De prevalentie van auto-immuun schildklieraandoeningen werd onderzocht in onze groep patiënten met achalasie en vergeleken met de prevalentie in de algemene bevolking. In ons cohort werd inderdaad een hogere prevalentie auto-immuun schildklierziekte gevonden (Odds Ratio 6,58 (3,42 – 12,66). Ook andere auto-immuun aandoeningen werden gerapporteerd. Deze bevindingen ondersteunen de auto-immuun hypothese. Een familiaire clustering, hetgeen ook vaak gebruikelijk is bij auto-immuun aandoeningen, kon niet worden gevonden. Dit is waarschijnlijk het gevolg van het feit dat het cohort relatief klein is om de familiaire clustering van een aandoening met een lage prevalentie te onderzoeken.

Concluderend is achalasie een zeldzame slokdarm motiliteits stoornis, die mogelijk een auto-immuun etiologie heeft aangezien auto-immuun schildklierziekten vaker voorkomen bij patiënten met achalasie. Deze observatie heeft echter niet geleid tot een oorzakelijk behandeling. De behandeling is nog steeds symptomatisch en gericht op het verlagen van de rustdruk van de onderste slokdarmsphincter. Pneumodilatatie is een effectieve therapie, hoewel jonge patiënten, patiënten met klassieke achalasie, patiënten met een hoge rustdruk in de onderste slokdarmsphincter 3 maanden na dilatatie en patiënten waarbij de taille van de ballon niet volledig verstrijkt tijdens dilatatie een slechtere respons hebben. In deze patiëntencategorie zal eerder gedurende de behandeling alternatieve therapie zoals laparoscopische myotomie moeten worden overwogen.

Patiënten met achalasie hebben een duidelijk verhoogd risico op het ontwikkelen van een slokdarmcarcinoom, welke vaak in een irresectabel stadium wordt gediagnosticeerd. Het is

van belang om de patiënten met het hoogste risico te definiëren omdat zij het meest gebaat zijn bij intensievere surveillance.

Lang bestaande ziekte, p53 overexpressie en matig tot ernstige ontsteking in surveillance biopten, voedselstase en ontwikkelen van Barrett's metaplasie bij surveillance scopie blijken risicofactoren te zijn. Onderzoek in de toekomst moet uitwijzen wat de beste surveillance methode en het beste interval is. Patiënten met risicofactoren zouden een waarschijnlijk jaarlijkse surveillance aangeboden moeten worden, 10 jaar na het ontstaan van de klachten. Om de opbrengst van deze scopie te optimaliseren moet de slokdarm goed schoon zijn, door het voorschrijven van een vloeibaar dieet gedurende 3 dagen voorafgaand aan de scopie.

In geval van voedselstase of ernstige ontsteking in de surveillance biopten moet hernieuwde behandeling (indien mogelijk) worden overwogen ook als patiënt geen evidente verslechtering aangeeft.

Om deze strategie te bewijzen is een prospectieve gerandomiseerde studie nodig wat moeilijk uit te voeren zal zijn. Het is echter belangrijk om patiënten met achalasie zoveel mogelijk te clusteren en te behandelen en vervolgen volgens een strikt protocol om het kankerrisico vast te stellen na nog langere follow-up en de uitkomsten van een intensievere surveillance en behandeling te onderzoeken.

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Curriculum vitae

Ivonne Leeuwenburgh werd geboren op 12 december 1971 te Brielle. In 1990 behaalde zij haar atheneum examen aan de Willem van Oranje scholengemeenschap te Oud-Beijerland. Aansluitend startte zij met haar studie geneeskunde te Rotterdam aan de Erasmus Universiteit. Het arts-examen werd in 1996 cum laude afgelegd. Vervolgens werkte zij als arts-assistente interne geneeskunde in ziekenhuis "De Baronie" in Breda. In 1999 startte zij hier met haar opleiding interne geneeskunde (opleider dr. P.J. Stijnen). Van 2001 t/m 2002 vervolgde zij haar opleiding interne geneeskunde in het Sint Franciscus Gasthuis te Rotterdam (opleider dr. H.S.L.M Tjen). In 2003 kreeg zij de mogelijkheid de opleiding tot maag- darm- en lever-arts op de afdeling MDL van het Erasmus Medisch Centrum te Rotterdam (opleider Prof. Dr. E.J. Kuipers) te volgen. Op 1 januari 2006 volgde registratie tot MDL-arts en sindsdien is zij werkzaam als MDL-arts in het Sint Franciscus Gasthuis te Rotterdam.

Gedurende de vervolgopleiding tot MDL-arts behandelde zij een groot cohort achalasie patiënten. Tevens werd onderzoek verricht uitmondend in dit proefschrift (promotor Prof. dr. E.J. Kuipers).

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dat we nu meer tijd samen zullen hebben is een utopie maar ik zal proberen het evenwicht meer in het midden te krijgen. En zullen we na jouw boekje dan eindelijk ons huis maar eens afklussen??

acetylcholinesterase = cholinesterase.

acetylcholinetransferase z.o. transferase.

acetylcoënzym A: het met de acetylrest CH_3CO verbonden coënzym A; fungeert als overdrager van acetylgroepen.

acetylering: het invoeren van een acetylgroep in een molecule.

Ac-G: accelerator globulin (E.): Ac-globuline, stollingsfactor V in het bloed (proaccelerine).

achalasia, -asie (Gr. a = niet; chalasis = ontspanning): het ontbreken van volledige relaxatie van glad spierweefsel, in het bijzonder van het maagdarmkanaal. — **a-ia cardiae**: na het slikken blijft de verslapping van de onderste „slokdarm-sfincter" (er is geen echte kringspier) uit, door *neuromusculaire dysfunctie; z.o. cardiospasmus.

acheilia, -eilie (Gr. cheilos = lip): aangeboren misvorming waarbij een of beide lippen ontbreken (niet verwarren met achylie).