Reflux disease and achalasia: Failure of the gatekeeper
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Chapter 13
Screening for dysplasia in idiopathic achalasia using Lugol staining
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ABSTRACT

Background and aims:
Patient with achalasia have a 10-50 fold increased risk to develop esophageal squamous cell carcinoma (ESCC). Early diagnosis of ESCC is essential, and detection of an earlier dysplastic stage is preferred. Endoscopic detection is however difficult and often delayed. Chromoendoscopy with Lugol dye increases detection rates dysplasia and ESCC to 91-100%. The aim of this study was therefore to evaluate a screening program using chromoendoscopy with Lugol to detect dysplasia in patients with idiopathic achalasia.

Methods:
A cohort of 138 patients with achalasia (86 males, mean age 50.4yr, range 20-87) underwent 3-annually upper endoscopy with Lugol dye staining. Suspected regions for dysplasia with white light endoscopy and unstained lesions after Lugol staining were biopsied. Patients with low grade intraepithelial neoplasia (LGIN) underwent yearly screening, while patients with high grade intraepithelial neoplasia or ESCC were treated endoscopically, surgically or by radiotherapy.

Results:
On a per patient basis, 2 of 138 patients (1.4%) developed ESCC in our cohort, leading to an incidence of 122/100.000 person-years. The hazard rate of esophageal cancer was 10 compared to the matched population. Dysplasia/ESCC was detected only after a median of 23.5 yrs (IQR 22-29yr) after achalasia was diagnosed. On a per-endoscopy basis, 56 and 76 suspicious lesions were identified during white light endoscopies (n=273) and after Lugol staining respectively. Biopsies taken from these lesions were positive for dysplasia and ESCC in 13 and 2 cases respectively. Lugol staining detected significantly more histologically proven cases of LGIN (n=13, 100%) compared to white light endoscopy (n=8, 62%, p<0.01). Patients with LGIN underwent annual control endoscopies: no progression to high grade dysplasia or ESCC was observed during a period of 4.6 yrs of follow-up. LGIN was confirmed in 9 control endoscopies of 4 patients.

Conclusions:
Patients with long-term disease (>15-20yr) have an increased risk to develop dysplasia and carcinoma. In these patients, the use of a screening program may be indicated, preferably using Lugol chromoendoscopy to detect early lesions.
INTRODUCTION

In achalasia, dysplastic changes are likely due to esophageal stasis of food and fluids caused by the functional obstruction of the distal esophagus. Increased bacterial growth and chemical irritation from the continuous decomposition of food and saliva can induce chronic hyperplastic esophagitis and eventually malignant transformation of esophageal epithelial cells. In addition, following treatment, reduced lower esophageal sphincter (LES) pressure combined with absence of peristalsis may promote prolonged acid exposure and thereby lead to esophagitis, Barrett’s esophagus and eventually adenocarcinoma. As a result, the risk of developing esophageal carcinoma is highly increased in patients with achalasia, ranging from 10- to 50-fold. In line, a recent large long-term prospective trial demonstrated a hazard ratio of 28 for developing esophageal squamous cell carcinoma (ESCC) in patients with achalasia compared to matched controls. In addition, we previously observed in a retrospective study of patients with longstanding achalasia that 19% of the deceased patients had died due to esophageal carcinoma.

As one of the main symptoms of esophageal carcinoma, i.e. dysphagia, is frequently attributed to recurrence or exacerbation of achalasia, the diagnosis of this long-term complication is often delayed. Since the prognosis of advanced esophageal carcinoma is very poor, early detection of dysplastic lesions, similar to Barrett’s esophagus, is essential. However, dysplastic lesions in the esophagus are difficult to detect using conventional endoscopy, especially in patients with achalasia due to hyperplastic esophagitis. Therefore, alternative screening tools should be applied. Chromoendoscopy with Lugol dye has proven efficacious in patients with a high prevalence of ESCC to detect precursor lesions such as high and low grade intraepithelial neoplasia with a sensitivity of 91-100%. Hence, we evaluated the presence of intraepithelial neoplasia and intestinal metaplasia in patients with longstanding achalasia using this technique.

METHODS

Patients

Following approval by the Institutional Review Board of the Academic Medical Center (Amsterdam, The Netherlands) and after obtaining patient written informed consent, 138 patients were invited to participate in the study. Achalasia was diagnosed based on the absence of peristalsis and relaxation of the LES (nadir relaxation pressure >10mmHg) on esophageal manometry. As we anticipated that mainly patients with longstanding achalasia would be at risk, patients were categorised based on the time since diagnosis of achalasia. The diagnosis of achalasia was made <10 years before study participation in 79 patients, 10-20 years in 31 patients and ≥20 years in 28 patients.
Treatment
Patients had been treated by pneumodilation (n= 92, 64%) or Heller myotomy (n= 28, 19%), or both (n=18, 13%). Pneumodilation was performed using a graded pneumodilation protocol as described previously (20). In short, dilations were performed with a Rigiflex balloon (Rigiflex, Nanterre, France) of 30 and 35 mm with at least 2 weeks between the two dilation sessions. In case of recurrent symptoms, pneumatic dilation was repeated with a 35 mm balloon or patients were referred to the surgeon. For Heller myotomy, the esophagogastric junction (EGJ) was approached laparoscopically and a myotomy was performed extending 6 cm above the EGJ and 1.5 cm over the stomach followed by a fundoplication according to Dor.

Design
Patients underwent upper endoscopy combined with Lugol staining. Endoscopies were performed after a liquid diet for 3 days and an overnight fast. When no abnormalities were found, the endoscopy was repeated after 3 years. Upper endoscopy was repeated one year later in case of low-grade intraepithelial neoplasia (LGIN). Patients with high-grade intraepithelial neoplasia (HGIN) or ESCC underwent endoscopic resection and/or ablation, surgery or radiotherapy.

Endoscopy
Patients were given local pharyngeal anaesthesia with lidocaine (Xylocaine). When requested, patients were sedated with midazolam (Dormicum). Upper endoscopy was performed using a flexible GIF160Q or GIF180Q gastroscope (Olympus Europe, Hamburg, Germany). After thorough inspection with white light suspicious lesions were noted. Subsequently, a spray catheter was inserted through the instrumentation channel. The esophagus was sprayed with acetylcysteine followed by distilled water. The esophagus was then stained from the upper esophageal sphincter to the Z-line with 20 ml Lugol’s iodine solution (2.5%) under direct vision. Excess dye was aspirated, the esophageal wall was rinsed with distilled water and the esophagus was re-inspected for poorly or unstained areas. (Figure 1.)

Subsequently biopsies were taken. In the first 30 endoscopies, random biopsies were taken subsequently only areas suspicious for dysplasia, as indicated by white light endoscopy, Lugol staining or both were biopsied. Biopsies were fixed in formaldehyde and sent for pathological evaluation.
Screening for dysplasia using Lugol’s staining

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Figure 1 | White light images (A and C) and the corresponding Lugol chromoendoscopy images (B and D) are demonstrated for 2 patients. In Figure A, a widened and elongated esophagus with hyperplastic squamous epithelium is observed. After Lugol staining (B), no unstained lesions are observed. In the second example, a comparably elongated and widened esophagus is observed (C), which is typically observed in patients with achalasia. In contrast, 2 unstained lesions are observed with Lugol chromoendoscopy (D), in which histopathological evaluation revealed LGIN.

Histopathological evaluation

Formalin-fixed biopsies were processed to hematoxylin and eosin stained slides for routine histological evaluation by a junior and senior GI-pathologists. Biopsies were evaluated for the presence of dysplasia according to the criteria of the World Health Organisation, defined as: no intraepithelial neoplasia, LGIN, HGIN, or ESCC. (21). Cases of LGIN were re-evaluated by an expert GI-pathologist (MV).
**Timed barium esophagogram**

Dysplastic changes are likely due to esophageal stasis of food and fluids. To determine the level of esophageal stasis, timed barium esophagograms were obtained. According to de Oliveira et al.,\(^2\) patients ingested the maximum tolerable amount of low density barium sulphate, without regurgitation or aspiration. With the patient upright in a slight left posterior position, a radiograph was taken at time = 0 and after 1, 2 and 5 min. The distance from the tapered distal esophagus to the top of the barium column was measured. The five min barium height is used as measure of esophageal emptying. The height is measured relative to the height of the vertebrae (level of the diaphragm).

**Statistical analysis**

Statistical analysis was performed using SPSS 16.0 (IBM Corporation, Somers, NY, United States). Data are presented as mean ± SEM when parametric, and as median (IQR) when not parametric. Data on the incidence of esophageal carcinoma in the general population of the Netherlands were obtained from the study of Leeuwenburgh et al.\(^2\)\(^2\) Parametric data were compared using a Student’s t-test. Comparisons of proportions were made using a Fisher’s exact test. All reported p-values are 2 tailed, and a value of 0.05 is regarded as statistically significant.

**RESULTS**

**Patients and follow up**

The cohort of patients consisted of 138 patients with achalasia (86 males (60 %)) with a mean age of 50 ± 1.2 years at the time of the first endoscopy (range 20-87 years). During the inclusion period (from 2000 to 2011), 5 patients were excluded due to pseudo-achalasia. The first endoscopy was performed 9.7 ± 0.8 years after diagnosis of achalasia (range 0-37 years). The diagnosis was made after a median of 3 (IQR 2-8) years after onset of complaints. A total of 273 endoscopies were performed with a median of 2 (range 1-8) endoscopies per patient. The median follow up time during the study was 32 months. Patients had a total of 1642 years of achalasia, and 2124 years of symptoms.

**Lesions**

Overall, carcinoma or dysplasia was detected in 6 patients (4.3%), of which 2 cases had ESCC (1.4%) and 4 were diagnosed with LGIN (2.8%). The incidence of ESCC therefore is 122/100.000 person-years. Compared to the incidence of 12/100.000 in the normal population\(^2\), the hazard ratio is 10 for ESCC in our total cohort of patients with achalasia.
Esophageal carcinoma developed a median of 23.5 yrs (IQR 22-29yr) after diagnosis, and mainly occurred >20 years after diagnosis. In the group from 0-10 years after diagnosis (n=73), 1 case of LGIN was detected during follow up, whereas no lesions were detected in the 10-20 yrs (n=31). In contrast, most lesions were detected in the > 20 years group (n=34) with 2 ESCCs and 3 cases of LGIN. This results in a prevalence of cancer or dysplasia of 14.7% in this subgroup. (Figure 2) The incidence for ESCC in the >15 years category is 519/100.000 person-years, which is 43 times higher than in the overall population.

Both ESCCs were detected during the initial screening endoscopy, 22 and 33 years after diagnosis. Both patients had no alarm signs such as worsening of dysphagia, haematemesis or weight loss. One patient was considered to have a superficially spreading tumour and underwent curative surgery (esophagectomy). Eight years later, this patient is still disease free. The other patient with ESCC had an invasive tumour and underwent palliative radiotherapy. This patient died 9 months after diagnosis. In both patients HGIN was detected in biopsies obtained during endoscopy surrounding the squamous cell carcinoma.

Low grade intestinal neoplasia was detected by routine endoscopy in 2 patients and by Lugol staining in 2 additional patients (4 in total). During a mean follow up of 55 months, LGIN was confirmed in 9 of 16 follow up endoscopies, at least once in each patient. No progression to HGIN or ESCC was observed.
White light vs. Lugol staining

To determine the additional value of Lugol staining we compared the detection rate of white light endoscopy and Lugol staining. In 138 patients, a total of 273 endoscopies were performed detecting 73 suspicious areas using white light. Adding of Lugol staining led to a further detection of 44 suspicious areas, or a total number of 117 suspicious lesions. (Table 1.)

Both cases of ESCC were detected by white light and Lugol staining. Detection of LGIN as confirmed by pathology was significantly improved by Lugol staining (13 of 13 lesions, 100%) compared to white light endoscopy (8 of 13 lesions, 62%) (p<0.01, Fisher’s Exact).

Histopathological evaluation of other unstained lesions revealed changes indefinite for dysplasia (3 lesions, 3%), inflammatory changes attributable to reflux (36 lesions, 31%), inflammatory changes of unknown cause (33 lesions, 28%), Barrett’s epithelium (2 lesions, 2%), candida esophagitis (5 lesions, 4%), hyperkeratosis (10 lesions, 9%) and no abnormalities in 13 unstained lesions (11%).

The sensitivity for detection of ESCC/dysplasia was therefore 67% (10 of 15 lesions) and 100% (15 of 15 lesions) for white light endoscopy and Lugol chromoendoscopy respectively. The positive predictive value was comparable with 14% (10 of 73 lesions) and 13% (15 of 117 lesions) for white light endoscopy and Lugol chromoendoscopy respectively.

<table>
<thead>
<tr>
<th>Esophageal lesions (no.)</th>
<th>Before Lugol</th>
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<td></td>
<td>Endoscopies (no.)</td>
<td>Endoscopies (no.)</td>
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<td>0</td>
</tr>
<tr>
<td>Total areas</td>
<td>73</td>
<td>117</td>
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</table>

Table 1 | The number of lesions per endoscopy with white light and Lugol staining are shown.

Risk factors

The most important risk factor for dysplasia was the duration of the disease or time after diagnosis. As emphasized, esophageal dysplasia or carcinoma developed a median of 23.5 yrs (IQR 22-29yr) after diagnosis, and mainly occurred >20 years after diagnosis.

Secondly, due to continuous irritation of the esophageal mucosa, esophageal stasis is assumed to precede dysplastic changes. In line, dysplastic changes occurred more often in patients with esophageal stasis during endoscopy compared to patients without stasis. (4 of 28 (14%) patients
vs. 2 of 97 (2.1%) patients, Fisher’s exact p=0.02). To determine the validity of stasis observed during endoscopy we compared it to the level of stasis on a timed barium esophagogram. As expected, patients with esophageal stasis during endoscopy also had significantly higher levels of esophageal stasis on timed barium esophagogram after 5 minutes (8.3 ± 1.3 cm vs. 3.0 ± 0.5 cm, p<0.0001).

Although alcohol consumption and tobacco use was not prospectively assessed in detail in all patients, only 1 patient with dysplasia or cancer had a history of tobacco use whereas none had a history of alcohol abuse.

DISCUSSION

In the present study, we evaluated the risk of esophageal dysplasia and carcinoma in patients with achalasia using Lugol chromoendoscopy. Dysplastic changes and ESCC occurred primarily in patients with longstanding disease (> 20 years), i.e. in 4.3 and 1.4% of this subgroup respectively. Using Lugol staining, the detection rate of LGIN significantly increased compared to white light endoscopy. Screening for dysplasia using Lugol staining might therefore be advocated in patients with longstanding disease.

Continuous irritation of the esophageal mucosa due to esophageal stasis or reflux of gastric contents is assumed to precede dysplastic changes. Subsequent hyperplastic or metaplastic changes are frequently observed in patients with achalasia, and eventually these epithelial changes can lead to malignant transformation. According to a recent meta-analysis by Eckardt et al, patients with achalasia have an increased risk for ESCC, with a hazard ratio of 10-50 compared to the normal population. We confirmed the increased prevalence of esophageal squamous cell carcinoma in our total cohort, demonstrating a hazard ratio of 10. Interestingly, ESCC only occurred after a median time of 26 years after the diagnosis of achalasia in our cohort. This time after diagnosis is in line with five earlier studies, in which the time between symptoms of achalasia and the diagnosis of ESCC varied from 24 to 32 years. The only study that failed to find an increased risk for squamous cell carcinoma had a mean follow up after diagnosis of only 6 years. The relatively low hazard ratio in our study may also be attributable to the large number of patients with 0-10 years of follow up after diagnosis (n=73, 53%). When only patients with >15 years of achalasia were analysed, the relative risk increases to 43 compared to the general population. Therefore, screening of patients with achalasia for dysplastic changes seems only indicated in patients with longstanding disease.
The prognosis of ESCC is mainly determined by the stage of disease at the time of detection.\textsuperscript{13,16} In achalasia, the prognosis of ESCC is considered to be even worse than in the general population, mainly attributed to the delayed diagnosis. Three main reasons can be proposed: first, most patients are used to having some residual degree of dysphagia even after treatment, masking the increase in dysphagia caused by the development of ESCC. Secondly, patients adapt to dysphagia and seek medical care at a much later stage. Finally, as the esophagus is often dilated, a malignant lesion must reach a substantial size before causing additional dysphagia.\textsuperscript{13,16} In line, both patients with ESCC in the current study were diagnosed in the screening program but did not report aggravating dysphagia or alarm symptoms.

Based on the delayed diagnosis and the bad prognosis of ESCC, early detection of dysplastic lesions becomes of crucial importance. This study demonstrated for the first time that staining with Lugol significantly improves the detection of LGIN in achalasia with a sensitivity of up to 100%. Previous studies have clearly demonstrated that the risk of ESCC is strongly dependent on the grade of dysplasia. For squamous LGIN, the risk to develop ESCC is 5% within 3.5 years and 24% within 13.5 years in a high-risk population in northern China, which is 2.9 times higher compared to the local general population.\textsuperscript{32,33} Although none of the patients with LGIN in our study showed progression to HGIN or ESCC, it should be emphasized that our follow-up period of 55 months is rather short. For HGIN, the risk to develop ESCC was 50-74% during a follow-up period of 13.5 years in a prospective study in the same high-risk population in China. Taken together, we conclude that Lugol staining improves detection of dysplastic lesions, which are important precursor lesions of ESCC.

Although the sensitivity of Lugol staining as screening tool is excellent (100%), a main disadvantage is the high false positive lesion rate.\textsuperscript{36} The positive predictive value of lesions observed after Lugol staining was only 13% for dysplasia or carcinoma in our study, mainly due to a high rate of reflux- and aspecific inflammatory changes causing unstained lesions. Moreover, the optimal screening interval remains to be determined. To avoid numerous screening endoscopies, ideally, patients should be stratified according to their risk to develop dysplasia and cancer. One potential approach could be histopathological evaluation of p53 immunoreactivity in the squamous mucosa. In patients with achalasia, overexpression of p53 was observed in 82% of patients with ESCC and 67% of patients with LGIN, and interestingly, even 6 years (range 1-11) prior to the diagnosis of ESCC or LGIN.\textsuperscript{23} Further studies involving p53 and other biomarkers such as Ki67 or p16 might therefore possibly be useful to stratify patients according to their risk to obtain ESCC.\textsuperscript{27} However, for now we would recommend to only screen patients with long-term achalasia on a 3-year basis.

Current guidelines do not propose regular screening for dysplasia or ESCC in patients with achalasia. A screening program is only indicated if early detection leads to adequate therapy and
a reduction in mortality or morbidity. Large studies in a high-risk population for ESCC in China demonstrated that screening and timely treatment reduced mortality of ESCC to the level that survival of patients is comparable to healthy controls. Based on our data, we propose to screen patients with > 15 years of achalasia. However, as our study is small and only has a limited follow-up period of 32 months, further studies are definitely needed to prove that screening in patients with achalasia can indeed increase survival.

In conclusion, patients with longstanding disease and esophageal stasis, the risk to develop esophageal dysplasia and carcinoma is significantly increased. As Lugol staining is more sensitive than standard endoscopy to detect dysplastic lesions, a screening program may including Lugol staining may be indicated. Further long-term follow-up studies are however needed to demonstrate its impact on survival, preferably in a multi-center setting.
REFERENCE LIST


