Long-term outcomes of the randomized controlled trial comparing 5-aminolaevulinic acid and Photofrin photodynamic therapy for Barrett’s oesophagus related neoplasia

Short title: Long-term outcomes of PDT therapy

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Conflict of interest:

The authors declare that they have no conflict of interest.

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ABSTRACT:

Objective:

Photodynamic therapy (PDT) was used as therapy for early neoplasia associated with Barrett’s oesophagus (BE). This is 5-year follow-up of patients enrolled into randomized controlled trial of 5-aminolaevulinic acid (ALA) vs. Photofrin PDT.

Methods:

Biopsies were taken from original Barrett’s segment during endoscopic follow up using Seattle protocol. Endoscopic mucosal resection (EMR) +/-radiofrequency ablation (RFA) was preferred therapy in patients who failed PDT and/or had recurrent neoplasia.

Results:

Fifty eight of 64 patients enrolled in the original trial were followed up including 31 patients treated with ALA PDT (17 patients with ≤6cm, 14 patients with >6cm segment of BE) and 27 treated with Photofrin PDT (14 patients with ≤6cm, 13 patients with >6cm BE). Initial success was achieved in 65% (20/31) ALA and 48% (13/27) Photofrin patients, (p=0.289). 35% patients (7/20) relapsed in ALA group and 54% (7/13) relapsed in Photofrin group (p=0.472). At a median follow-up of 67 months, no significant difference was found in long term complete reversal of intestinal metaplasia (CR-IM) and complete reversal of dysplasia (CR-D) between ALA and Photofrin groups (78% vs 63%; p=0.18; 90% vs 76%; p=0.26). Original length of BE did not alter long-term outcome. Four patients from each group progressed to invasive oesophageal adenocarcinoma. Initial success of ALA PDT was associated with significantly better likelihood of long-term remission (p=0.03).

Conclusions:
Initial response to PDT plays key role in long term outcome. RFA +/- EMR have, however, become preferred minimally invasive ablative therapy for BE-related neoplasia due to poor efficacy of PDT.

**Key words:** Barrett’s oesophagus, endoscopic mucosal resection, photodynamic therapy, radiofrequency ablation
INTRODUCTION:

The incidence of oesophageal adenocarcinoma (OAC) has increased rapidly in the Western countries in the last few decades and the 5-year survival rate remains still poor at only 15% [1]. Barrett’s esophagus (BE) represents a pre-malignant condition leading to OAC [2]. Until recently, oesophagectomy was standard therapy for both invasive OAC and early neoplasia arising in BE including intramucosal carcinoma and high grade dysplasia [3]. Surgery performed even for early BE related neoplasia is associated with significant morbidity and there has been rapid progress of minimally invasive therapy in the last two decades [4-7]. Photodynamic therapy using different photosensitizers including porfimer sodium (Photofrin) [4,8], 5-aminolaevulinic acid (ALA) [9,10] or m-tetrahydroxyphenyl chlorine [11] has been investigated as a therapeutic option. Nevertheless, due to poor efficacy of PDT [8] and side effects (especially related to Photofrin) [4,8], radiofrequency ablation (RFA) in combination with endoscopic mucosal resection (EMR) have become preferred, first line endoscopic therapy for BE-related early neoplasia within the last few years [5,12-14].

This single-centre study commenced before RFA was available and aimed to establish the relative merits of Photofrin and ALA PDT to treat BE-related neoplasia (high grade dysplasia (HGD) or intramucosal carcinoma (IMC)) [8]. The current paper reports on the long-term outcomes of this trial and specifically examines the outcomes of additional therapy in patients who failed PDT or who developed recurrent disease.
METHODS:

Patients:

A total of 64 patients were enrolled into the randomized controlled trial between 2006 and 2009 at University College London Hospital (UCLH). Thirty-four patients were photosensitised with ALA and 30 with Photofrin. A maximum of 2 treatment sessions, 3 months apart, were allowed for Photofrin and 3 treatment sessions were allowed for ALA. ALA (DUSA Pharmaceuticals, NY, USA) was administered orally on the morning of the procedure at the dose of 60 mg/kg and Photofrin (Axcan Pharma) was administered 3 days prior to the treatment by intravenous injection into a large vein in the ante-cubital fossa at a dose of 2 mg/kg. Light precautions were followed and a light metre was provided. Light was delivered by either an 18-mm transparent plastic balloon for ALA (supplied by DUSA) or a bolster device for Photofrin (made by CAM, UCL). Great care was taken to ensure that there was no overlap when using Photofrin-PDT (for the high-potential risk of stricture formation). For both, ALA-PDT and Photofrin-PDT, red light (635nm) was delivered [8].

All patients treated with PDT received PPI before and after PDT. Since the current study is a long-term follow up of treatment, 3 patients from each group were excluded as no follow up biopsies were taken. In the ALA group, one patient was followed-up locally and no histology results are available; one patient developed invasive moderately differentiated adenocarcinoma between enrolment and initial PDT and underwent oesophagectomy; one patient had poorly differentiated adenocarcinoma at time of PDT and underwent chemoradiotherapy. In the Photofrin group, one patient died 10 days after PDT from Clostridium difficile infection; one patient had invasive moderately differentiated carcinoma at time of PDT and underwent oesophagectomy and one patient died 4 weeks after PDT from a myocardial infarction. This patient was known to suffer from aortic stenosis.
The patients were originally stratified by the number of treatment segments needed. A maximum of 6cm could be treated in each segment. A total of 17 patients from ALA group had a single treatment segment and 14 patients had a double segment (>6cm) of BE treated. A total of 14 patients from the Photofrin group had a single segment treatment of BE and 13 patients had a double segment treatment. All patients had high grade dysplasia in Barrett’s oesophagus when enrolled into the original randomised controlled trial. The follow-up data for this paper were censored in August 2016.

**Follow-up protocol:**

Four quadrant large-cup biopsies were taken every 2 cm throughout the original Barrett’s segment at 6 weeks, 4 and 12 months and then annually after completion of treatment, unless the discovery of persistent or recurrent neoplasia required further intervention. High-resolution white-light endoscopy combined with either narrow-band imaging (Olympus) or i-Scan enhancement (Pentax) was used in follow-up procedures. The Prague classification was used to assess BE and the revised Vienna classification was used for histological assessment of neoplasia [15,16].

**Subsequent therapy**

If BE-related neoplasia persisted despite PDT or recurred later, appropriate therapy was chosen: endoscopic mucosal resection (EMR) was performed if nodular non-invasive neoplasia was present; radiofrequency ablation (RFA) was used for flat dysplasia, in line with standard protocols [5]. Surgery and/or chemoradiotherapy was reserved for those with invasive (submucosally invasive and more advanced) cancer.
Declaration:

The original randomised controlled trial of PDT was registered at: http://www.controlled-trials.com, number ISRCTN16444200 and approved by Berkshire Research Ethics Committee 13/1/2006 (ref: 05/Q1602/193). RFA data come from the UK RFA registry, number ISRCTN93069556. National Research Ethics Service (NRES) (ref. 08/H0714/27) approved the RFA registry - date applied: 12/06/2013 and date assigned 14/6/2013.

Written, informed consent was obtained from each patient included in the study. The procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Statistics:

Data obtained were tested statistically by means of descriptive statistics, Fisher’s exact test and Mann Whitney test. Log-rank analysis was undertaken using Kaplan-Meier plots of disease-free survival. All analysis was performed using Statistica software, version 13, 2013, Tulsa, OK, USA.

RESULTS:

Descriptive statistics:

There was no statistical difference in age between the two groups tested at the time of randomisation: ALA: mean 69+/-9, Photofrin: mean 67 +/- 9; p=0.41. No difference in length of Barrett’s oesophagus [cm] between the groups was found: ALA: median 6, IQR (interquartile range): 4-8; Photofrin: median 4, IQR: 3-9; p=0.9.

Median follow-up after completion of PDT treatment was 67 months (2-111 months).
Results at completion of PDT:

At the first endoscopy after completion of PDT, a statistically significant difference in CR-IM (complete reversal of intestinal metaplasia) was found between the groups, with ALA being more successful: ALA: 17/31 (55%) vs. Photofrin: 6/27 (22%); p=0.016. No difference was, however, found in CR-D (complete reversal of dysplasia) between the groups: ALA: 20/31 (65%) vs. Photofrin: 13/27 (48%); p=0.289.

Recurrence of Dysplasia after PDT

Seven of the 20 patients (35%) who were initially successfully treated with ALA and 7 of 13 with initial success after Photofrin (54%) had recurrence of dysplasia. Early and late recurrences occurred at similar frequencies after both ALA and Photofrin, anywhere between 4-109 months after completion of treatment. Long-term success (complete reversal of dysplasia) of PDT as a single treatment modality is shown in Figure 1. The Kaplan Meier curve demonstrates a 68% success for ALA-PDT at 5 years compared to 60% for Photofrin. The difference did not reach statistical significance (Log rank p=0.428).

Figure 2 shows the long-term success (CR-D) for all 58 patients when those who suffered a recurrence were treated with a non-PDT therapy. No statistically significant difference was found in final CR-D between the groups: the Kaplan Meier curve demonstrates a 90% success for ALA-PDT at 5 years compared to 76% for Photofrin, (Log rank p=0.26). Final CR-IM was also similar between the groups. At 5 years, the KM curve gave a predicted CR-IM rate of ALA: 78% vs. Photofrin: 63%, Log rank p=0.18 (Figure 3).
Outcomes at 5 year follow up depending on original length of BE:

No differences in CR-IM and CR-D were observed between patients with original single segment (≤6cm) and double segment (>6cm) treatment, whether they were treated with ALA or Photofrin. Details are shown in Table 1.

Outcomes depending on original success of PDT:

A total of 13 ALA patients (13/31; 42%) and 6 Photofrin patients (6/27; 22%); p=0.161, did not have recurrence of dysplasia and did not receive any further therapy. Long-term success of PDT as mono-therapy is shown in Figure 1. For those who failed and were treated with newer endoscopic therapy (EMR +/- RFA), long-term remission was also achieved in many. The final outcome of these patients is shown in Figure 4. Overall, initial success predicted long-term remission (p=0.045). This success remained statistically significant in the ALA group alone (p=0.03), but not in the Photofrin group alone (p=0.62).

Management of Recurrent Disease or failed PDT

Six recurrences after ALA PDT were treated with a combination of EMR and RFA, one was treated with radiotherapy for moderately differentiated invasive oesophageal adenocarcinoma. Six patients (86%) remain in remission long-term. The other has residual dysplasia.

Six recurrences after Photofrin were treated with a combination of EMR and RFA and one did not receive any therapy. Four patients (57%) remain in long-term remission, three have residual dysplasia.

The long-term outcomes of those who failed initial treatment are shown in Table 2.
**Progression to invasive cancer:**

Eight patients (8/58; 14%) progressed to invasive cancer. Four patients belonged to the ALA group (all of them progressed despite endoscopic therapy; subsequently, one was treated with surgery and chemotherapy, two with chemoradiotherapy and one with radiotherapy), four patients belonged to the Photofrin group (one patient, treated endoscopically, died of metastatic oesophageal adenocarcinoma; two patients underwent oesophagectomy (one after previous EMR); one received endoscopic therapy, but was not fit for suggested palliative radiotherapy due to psychological conditions). Figure 5 shows time and probability of development of invasive cancer in ALA and Photofrin group. At 5 year follow up, the KM probability of developing invasive cancer was similar in both treatment groups at just below 20%, following multimodality treatment (p=0.788).

**DISCUSSION:**

The aim of this study was to assess the long-term outcomes of patients with BE associated neoplasia who were originally enrolled into the randomised controlled trial comparing the photodynamic therapy drugs ALA and Photofrin [8].

At a median of more than 5 years follow-up, many patients who had entered remission initially relapsed and required further endoscopic intervention. This resulted in no statistically significant difference in CR-IM and CR-D between the groups treated with ALA or Photofrin. This differs from the situation at completion of PDT when CR-IM was significantly higher in ALA group. The explanation for this is clear: all failures of PDT and recurrences of dysplasia were treated in the same way: non-invasive neoplasia was treated endoscopically, usually by EMR +/- RFA (a minority of lesions was also treated with YAG laser or argon plasma.
coagulation), invasive neoplasia was treated with chemoradiotherapy and/or surgery (minority of cases). Our group has documented previously, that RFA in combination with EMR is effective, not only in BE ablation-naive patients [5,17], but also in those for whom photodynamic ablative therapy failed [18].

When analysing the effect of multimodality treatment, there were no differences in CR-IM and CR-D between shorter and longer segments of BE. This again differs from the situation at the originally reported median follow-up of two years post-PDT. At that time, patients with segments of dysplastic BE <6cm in length, treated with ALA had significantly higher CR-HGD compared to patients with longer segment treated with ALA [8]. The most likely reason is that RFA has a very high success rate for non-nodular dysplastic BE of any length.

Our data show that patients treated with ALA PDT which led to reversal of dysplasia had a significantly better long-term outcome when compared to those who failed PDT originally. This finding is not replicated in the Photofrin group. This is a crucial finding and confirms the fact, that Barrett’s oesophagus is not just one disease, which responds to any ablative therapy in a uniform way. Our original study allowed up to 3 consecutive PDT treatments. We previously reported that if dysplasia was not cleared after the first treatment, subsequent PDT was less likely to lead to remission [8]. Prasad et al looked at the biomarkers of patients with HGD or IMC in BE who underwent PDT with Photofrin. Loss of biomarkers related to progression of neoplasia in BE was associated with histologic downgrading of dysplasia after PDT; those patients with persistent positivity of biomarkers were at higher risk of recurrent HGD [19]. They confirmed in another study, that p16 allelic loss predicted decreased response to PDT [20]. Recently, Timmer et al reported, that genetic biomarkers can predict achievement of CR-D after endoscopic therapy and that patients with multiple genetic alterations may have a lower response rate [21]. Investigation of biomarkers could therefore
help in the management of dysplastic BE. We have similarly demonstrated that relapse is related to persistence of aneuploidy after treatment within the residual Barrett’s segment [22]. We have also demonstrated that pro-tumorigenic mutations can be found in post-ablation squamous mucosa as well as in mutant deep oesophageal glands; both are associated with dysplasia recurrence [23]. These findings all suggest that the genetic milieu of the residual Barrett’s segment is more complex and other abnormalities occur prior to the onset of dysplasia, a phenomenon that has been described in detail by a number of groups [24,25].

CR-D at completion of PDT was 65% in ALA group and 48% in Photofrin group. Most of these patients went on immediately to endoscopic rescue therapies. Despite multiple recurrences, which were treated mainly with RFA +/- EMR, CR-D at a median follow-up of 67 months has reached 77% in ALA group and 63% in Photofrin group. Therefore, in agreement with recent studies, combination of RFA +/- EMR seems to be an effective, durable and safe ablative therapy for dysplastic BE, even in those previously treated with PDT. The findings in this paper lend further support to the notion that EMR and RFA are better treatment options for patients with dysplastic BE. RFA is also associated with less frequent buried metaplasia (1%) when compared to PDT (14%) and therefore possible subsequent development of sub-squamous neoplasia should be less likely [26]. In conclusion, PDT alone does not offer a valuable long-term treatment for dysplastic Barrett’s oesophagus. Endoscopic treatment should continue to focus on newer treatment modalities.

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COMPETING INTERESTS:

The authors declare that they have no conflict of interest.

References:


5. Haidry RJ, Dunn JM, Butt MA, Burnell MG, Gupta A, Green S et al. Radiofrequency ablation and endoscopic mucosal resection for dysplastic barrett's esophagus and early


Figure 1: Long-term success (complete reversal of dysplasia) of PDT as a single treatment modality; $\chi^2 = 0.629$, p=0.428
Figure 2: Long-term success for all 58 patients (including those with PDT failure, who were treated with non-PDT endoscopic modalities subsequently): probability of CR-D; $\chi^2 = 0.964$, $p=0.26$. 
Figure 3: Long-term probability of CR-IM for all 58 patients; $\chi^2 = 1.237$, $p=0.18$. 
<table>
<thead>
<tr>
<th></th>
<th>CR-IM</th>
<th>CR-D</th>
</tr>
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<tbody>
<tr>
<td>ALA ≤6cm</td>
<td>12/17 (71%)</td>
<td>13/17 (76%)</td>
</tr>
<tr>
<td>ALA &gt;6cm</td>
<td>9/14 (64%)</td>
<td>11/14 (79%)</td>
</tr>
<tr>
<td>Photofrin ≤6cm</td>
<td>7/14 (50%)</td>
<td>8/14 (57%)</td>
</tr>
<tr>
<td>Photofrin &gt;6cm</td>
<td>6/13 (46%)</td>
<td>9/13 (69%)</td>
</tr>
</tbody>
</table>

**Table 1:** Detailed analysis of CR-IM and CR-D of each segment of BE treated with ALA or Photofrin PDT at 5-year follow-up.
<table>
<thead>
<tr>
<th>Initial Rx</th>
<th>No failed</th>
<th>F/U Rx modality</th>
<th>No treated</th>
<th>Long term remission</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA</td>
<td>11</td>
<td>No intervention</td>
<td>2</td>
<td>1</td>
<td>1 residual dysplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EMR and/or RFA</td>
<td>6</td>
<td>3</td>
<td>3 residual dysplasia</td>
</tr>
<tr>
<td>Surgery + chemotherapy</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>1 died of metastatic disease</td>
</tr>
<tr>
<td>Chemoradiotherapy</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>1 residual dysplasia</td>
</tr>
<tr>
<td>Photofrin</td>
<td>14</td>
<td>No intervention</td>
<td>1</td>
<td>0</td>
<td>1 residual HGD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EMR and/or RFA</td>
<td>10</td>
<td>7</td>
<td>2 residual dysplasia, 1 died of metastatic disease</td>
</tr>
<tr>
<td>Oesophagectomy</td>
<td>3</td>
<td>No follow-up biopsies</td>
<td></td>
<td></td>
<td>No follow-up biopsies</td>
</tr>
</tbody>
</table>

**Table 2:** The long-term outcomes of those who failed initial PDT treatment.
Figure 5: Long-term probability of development of invasive cancer: \( \chi^2 = 0.072, p=0.788 \).