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ORIGINAL ARTICLE

Durability of radiofrequency ablation for treatment of esophageal squamous cell neoplasia: 5-year follow-up of a treated cohort in China

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Background and Aims: Radiofrequency ablation (RFA) is an accepted treatment for flat Barrett's neoplasia. Less is known about RFA for esophageal squamous cell neoplasia (ESCN). Our group has reported several prospective studies of RFA for ESCN in China with promising results through 12 months of follow-up. In this cohort study we aimed to evaluate longer term outcomes after RFA for ESCN.

Methods: Patients with flat unstained lesions (USLs) on Lugol's endoscopy containing moderate-/high-grade intraepithelial neoplasia (MGIN/HGIN) or mucosal cancer were treated with RFA every 3 months until complete remission (CR; no MGIN or a worse histologic grade). Patients with CR at 12 months (CR12) were included for follow-up and underwent annual Lugol's endoscopy with biopsy sampling and re-RFA for flat USLs. The clinical course of patients with persistent ESCN at 12 months (treatment failures) is also reported.

Results: Among the 78 patients in CR12, 67 (86%) had sustained CR during a median of 48 months (interquartile range, 48-48) of follow-up and 5 endoscopies (interquartile range, 4-6). Recurrence occurred in 7 of 78 patients (9%; MGIN, n = 6; HGIN, n = 1); all lesions were managed with RFA. Four other patients (5%) had progression (to HGIN, n = 1; submucosal esophageal squamous cell carcinoma, n = 3). During follow-up protocol violations occurred in 46 of 78 patients (59%). Of the 12 treatment failures, progression occurred in 6. Overall, 2 patients developed subepithelial disease that was not visible after Lugol's endoscopy. Based on post-hoc analysis, the pink-color sign at baseline (a pink color change after Lugol's endoscopy) significantly predicted failure after RFA.

Conclusions: RFA is relatively easy to apply and can efficiently treat large areas with ESCN. Despite protocol violations that may have interfered with the efficacy of RFA in 59% of patients, most patients with CR12 had sustained CR during follow-up. However, some patients progressed to advanced disease and 2 developed subepithelial disease, not visible after Lugol's endoscopy. Based on currently available data, we advise the restriction of the use of RFA for flat MGIN and HGIN without the pink-color sign on Lugol's chromoendoscopy. (Clinical trial registration number: NCT02047305.) (Gastrointest Endosc 2018;■:1-13.)

(footnotes appear on last page of article)

Esophageal cancer is the eighth most common cancer worldwide, with a poor 5-year overall survival rate of 10% to 15%.[1] Globally, over 80% of esophageal cancers occur in developing countries, where nearly all cases are esophageal squamous cell carcinoma (ESCC). China has an especially high burden of disease; almost half of all ESCC cases in the world occur in China, where ESCC is the fourth leading cause of cancer-related death.[1,2]

When ESCC is diagnosed at a symptomatic stage, patients have a poor prognosis, because most cases are already locally advanced and/or have metastasized. The prognosis is excellent, however, when ESCC is diagnosed at an early stage when the neoplasia is confined to the mucosal layer. In these cases, curative endoscopic treatment can be performed with preservation of the esophagus. This mucosal neoplasia is generally asymptomatic but can be detected during screening endoscopy with the use of Lugol's chromoendoscopy. Endoscopic screening programs are widely implemented in high-risk areas in China, with more than 200,000 screening endoscopies being performed each year. ESCC and its precursor lesions are detected in about 3% of these screening endoscopies.[3]

The development of ESCC is a gradual process, starting with intraepithelial esophageal squamous cell

neoplasia (ESCN). In China, precursor lesions are classified in 3 progressive stages according to the proportion of the epithelial layer containing neoplasia. Low-grade, moderate-grade (MGIN), and high-grade intraepithelial neoplasia (HGIN) can be distinguished histologically, with one third, two thirds, and three thirds, respectively of the epithelial layer showing nuclear atypia, loss of normal cellular polarity, and abnormal tissue maturation.[4] MGIN and HGIN are considered indications for treatment given their progression rate to cancer (50% and 74%, respectively, over 13.5 years), whereas surveillance is indicated for low-grade intraepithelial neoplasia.[4,5]

Endoscopic treatment options include endoscopic resection (ER), either EMR or endoscopic submucosal dissection (ESD), or ablation techniques such as radiofrequency ablation (RFA). ER enables en-bloc resection, thereby allowing adequate pathologic assessment to evaluate the prognosis and the potential need for additional treatment. However, ER is technically demanding and has a risk for adverse events.[6] Most high-risk areas for ESCC have only limited endoscopic resources and expertise available, and therefore additional safe and effective treatment modalities of lower complexity are required. In addition, widespread ER may be associated with esophageal stenosis[6] and/or residual ESCN next to the ER scar.[7,8] Ablation techniques may offer theoretical advantages in selected patients, such as more widespread and complete eradication of oncogenic abnormalities, which should result in lower local recurrence rates and lower rates of esophageal stenosis.[9]

In 2008 we initiated a prospective trial to assess the safety and efficacy of RFA for eradicating ESCN in patients with flat type MGIN, HGIN, and early ESCC.[10,11] A complete remission (CR; absence of MGIN or a worse histologic grade in biopsy specimens) was established in 84% of patients at 12 months, with strictures occurring in 21%. After evaluation of several different circumferential RFA regimens, a single application of 12 J/cm^2 after application of Lugol's chromoendoscopy emerged as the favored regimen, resulting in a CR at 12 months (CR12) of 82% and a stricture rate of 6%. This study, however, reported only a short-term follow-up duration of 12 months. The long-term durability of RFA for ESCN is therefore still unknown. The current study is a continuation of the aforementioned trial and is the first to evaluate long-term durability of endoscopic RFA for eradicating MGIN, HGIN, and early flat ESCC for up to 5 years after baseline RFA treatment.

METHODS

The original 12-month study (clinicaltrials.gov identifier NCT02047305) was conducted between October 2008 and October 2011 at the Cancer Institute and Hospital, Chinese Academy of Medical Sciences in Beijing, China.[10,11] The present study was an extension of this study and was conducted between October 2009 and October 2016. The protocol was approved by the Cancer Institute and Hospital, Chinese Academy of Medical Sciences Institutional Review Board, and a written informed consent was signed by each patient. All authors had access to the study data and reviewed and approved the final manuscript.

Patients

Patients included in the original study were required to have at least 1 unstained lesion (USL) on Lugol's endoscopy, which measured ≥3 cm in length, covered ≥25% of the esophageal circumference, and contained flat MGIN, HGIN, or mucosal ESCC (ESCC-m). Multiple USLs were allowed as long as the total USL-bearing esophagus was ≤12 cm in length. EUS and CT were performed for patients with HGIN or ESCC-m to exclude submucosal invasion and/or lymphadenopathy. Other exclusion criteria were the presence of any nonflat lesions (lesions other than Paris type 0-IIb), esophageal strictures, and previous ablation therapy or ER. Further details of patient selection have been described previously.[11]

All patients with a CR, defined as the absence of MGIN, HGIN, or ESCC-m in biopsy specimens at 12 months (CR12), were included in this study extension. Patients with persistent ESCN at 12 months (treatment failures) were excluded. Other exclusion criteria were noncircumferential RFA at baseline and discontinuance of follow-up before 12 months.

Protocol treatment phase (the first 12 months)

The treatment protocol for the original study has previously been described in detail.[11] In summary, the USL-bearing esophagus was treated with 1 of 4 circumferential RFA regimens (BARXX^{360} system; Medtronic, Sunnyvale, Calif, USA) at baseline (Supplementary Table 1, available at www.giejournal.org), and sustainable tattoos were placed at the most proximal and distal edges to identify the treatment area (TA). Subsequently, patients underwent endoscopies at 3-month intervals with biopsy sampling and focal RFA (BARRX^{90}, 3 × 12 J/cm^2; Medtronic) for persisting USLs, until CR was achieved. All patients underwent a 12-month endoscopy with Lugol's staining and biopsy sampling to assess the primary endpoint of the original study (ie, the proportion of patients in CR12).

Protocol follow-up phase (from 12 to 60 months)

The follow-up protocol consisted of annual (± 3 months) follow-up endoscopies from 12 to 60 months after baseline. The TA was carefully evaluated by means of high-resolution endoscopy, narrow-band imaging, Lugol's staining (1.25%), and histologic analysis of 2 biopsy specimens per 2 cm of the TA. Flat type USLs in the TA were also biopsy sampled and treated with focal RFA. If subsequent

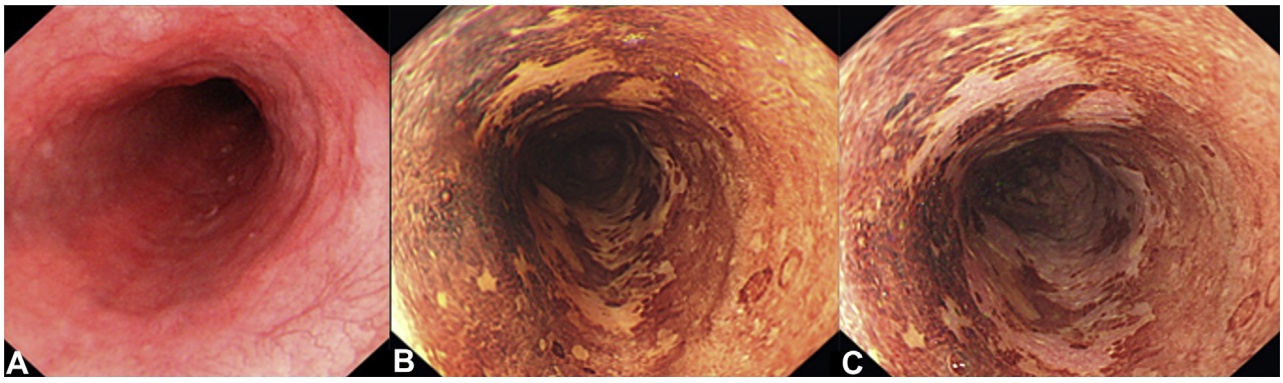

Figure 1. A patient with the pink-color sign after Lugol's staining. **A,** White-light endoscopy image shows no visible abnormalities. **B,** Directly after Lugol's staining a large yellow unstained lesion appeared from 3 to 7 o'clock and another unstained lesion more proximally from 10 to 12 o'clock. **C,** Several minutes after application of Lugol's, the entire unstained lesion turned pink.

biopsy samples showed MGIN or worse, reiterative RFA (re-RFA) treatment was repeated every 3 (± 1) months until CR was re-established. Patients with nonflat lesions (ie, lesions other than Paris type 0-IIb) or lesions with invasive cancer in biopsy specimens were treated with escape therapy according to institution's standards of care. If CR was re-established after escape treatment, the regular follow-up protocol with endoscopies on an annual basis was resumed. Outside of this formal follow-up study, we also followed the treatment failures at 12 months, which were treated and followed per investigator's discretion, realizing that both treatments and follow-up protocols varied in these patients and were not the same as those who achieved CR12.

Based on progressive insight on the significance of the "pink-color sign" (PCS) after Lugol's staining (ie, a color change after Lugol's staining from an initial whitish-yellow color to a pink color 2-3 minutes later), we performed a post-hoc assessment of all baseline images to score the presence of a PCS (Fig. 1).[12] Missing data for this study were retrospectively completed by review of medical records, and all data were monitored by a study coordinator and research fellow from Academic Medical Centre, Amsterdam, the Netherlands.

Histopathology examination

The histologic analysis of specimens during the original 12-month study has previously been described in detail.[10,11] All biopsy specimens obtained during the current follow-up study were routinely processed and reviewed by an expert GI pathologist (L.X. or N.L.), with review of selected specimens by a second expert (S.S.M.D.). All specimens were scored as no intraepithelial neoplasia, low-grade intraepithelial neoplasia, MGIN, HGIN, ESCC-m, or ESCC with invasion deeper than the mucosa,[13] with the most advanced result determining the histology status of the patient. All ER and surgical specimens in the study were reviewed by 1 of the expert GI pathologists for depth of ESCC invasion, the presence of ESCC at the deep or lateral resection margins, the grade of ESCC differentiation, and the presence of lymphovascular invasion.

Outcome measures

The primary endpoint of our follow-up study was the proportion of CR12 patients with sustained CR (defined as the absence of MGIN or worse in all TA biopsy samples) throughout the 48-month follow-up. Secondary study outcomes were (1) the proportion of patients with recurrent disease in the TA, defined as flat lesions with MGIN or a worse histologic grade and with a lower or equal histologic grade than the histologic grade at study entry; (2) the proportion of patients with progressive disease in the TA, defined as any nonflat lesion or detection of a more severe histologic grade than the histologic grade at study entry; (3) the occurrence of adverse events, including perforation, infection, bleeding requiring transfusion, stricture requiring dilatation, or death; and (4) the proportion of patients with the development of ESCN outside the TA.

Statistical analyses

Data analysis was performed using the IBM SPSS statistical software package (SPSS Inc, Chicago, Ill). Means with standard deviations were computed for normally distributed variables and medians with interquartile ranges (IQRs) for variables with a skewed distribution. Categorical variables were presented as frequencies and percentages of the total. Continuous and categorical variables were compared using the Student t test and Fisher exact test, respectively. All tests were 2-sided, and $P < .05$ was considered to be statistically significant.

The durability of CR was assessed using Kaplan-Meier survival curves. All patients in CR12 were included for this analysis, and "time zero" was the 12-month endoscopy. Using Cox regression, we conducted univariate analysis to assess patient characteristics possibly associated with recurrent or progressive disease. All variables with $P < .3$ were subsequently combined in multivariate Cox analysis,

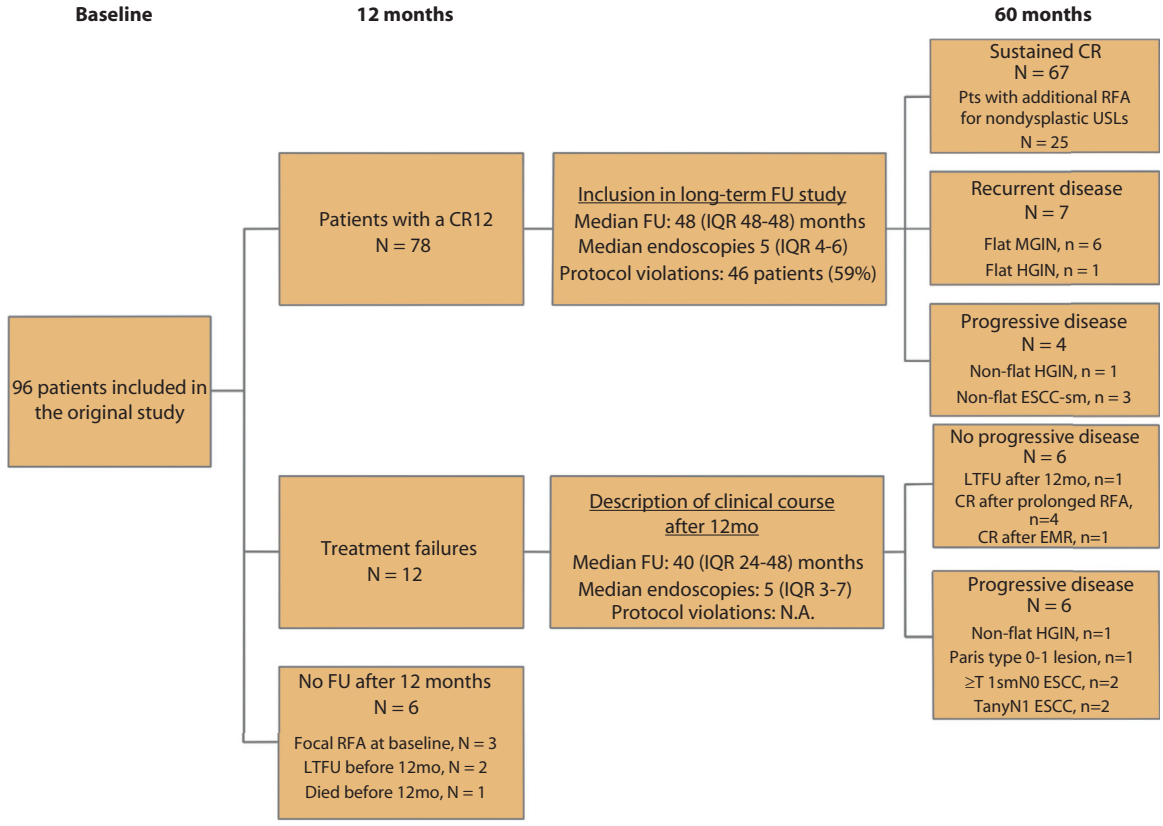

Figure 2. Patient flow during the full 5 years of treatment and follow-up. *CR*, Complete response, defined as absence of MGIN or worse; *CR12*, CR at 12 months endpoint of the original study; *ESCC*, esophageal squamous cell carcinoma; *ESCC-sm*, submucosal ESCC; *FU*, follow-up; *HGIN*, high-grade intraepithelial neoplasia; *IQR*, interquartile range; *LTFU*, lost to follow-up; *MGIN*, moderate-grade intraepithelial neoplasia; *RFA*, radiofrequency ablation.

and the variable with highest *P* value was excluded until only variables with $P < .05$ in multivariate analysis persisted. Additional analyses were performed to identify predictors for RFA failures in the total study population (ie, all 90 patients who were initially treated with circumferential RFA and were followed ≥12 months), including both patients who failed to achieve a CR12 and patients who had a CR12 but developed recurrent or progressive disease during the 48-month follow-up period as RFA failures.

RESULTS

Patients

Ninety-six patients were included in the original study, of which 78 were eligible for inclusion in the current follow-up study. Six patients from the original study were excluded: 3 patients with focal RFA at baseline and 3 with discontinued follow-up before 12 months (lost to follow-up [n = 2] or unrelated death [n = 1]). The 12 treatment failures at 12 months were not formally included in this follow-up study, yet the clinical course was assessed retrospectively (Fig. 2). The baseline diagnosis of the 78 included patients was MGIN (n = 39), HGIN (n = 33), or ESCC-m (n = 6), and the mean (± standard deviation) USL length at baseline was 6.1 (± 2.9) cm (range, 3-15) (Table 1).

During a median follow-up of 48 months (IQR, 48-48) after the 12-month endpoint of the original study, patients underwent a median of 5 endoscopies (IQR, 4-6). Sixty-seven patients (86%) completed the full 5-year follow-up of the study. The other 11 patients (14%) discontinued follow-up because of unrelated death (n = 3) or unrelated comorbidity (n = 2) or were lost to follow-up (n = 6). The median follow-up of these 11 patients was 24 months (IQR, 0-36).

Protocol violations (PVs) occurred in 46 of 78 patients (59%) and were categorized into 3 types: prolonged follow-up intervals exceeding 12 (± 3) months (PV-1), USLs in the TA that were left untreated (PV-2), and inadequate follow-up after treatment of recurrent ESCN (PV-3). Each type of PV (PV-1, PV-2, and/or PV-3) could occur at each follow-up endoscopy, so each PV could occur more than once in a single patient. Prolonged follow-up intervals (PV-1) occurred in 24 patients (31%), with a median delay of 8 months (IQR, 5-9) (Table 2). In 2 and 1 patients, respectively, the following 1 or 2 endoscopies were again delayed, and in the remaining 21 patients subsequent follow-up was performed as per protocol. In 30 patients (38%) USLs in the TA were sampled, but no direct RFA was performed (PV-2). In all cases the USL was deemed as inflammatory and not suspicious for ESCN based on the endoscopic appearance; however, in cases where MGIN or worse was found, an additional RFA treatment was

TABLE 1. Characteristics, findings, and treatments of the 78 patients in the follow-up study

Characteristics, findings, and treatments	Value
Protocol treatment phase (first 12 mo)	
Male gender	41 (53)
Mean age, y (± SD)	59.6 ± 6.6
Worst pathology grade at baseline	
MGIN	39 (50)
HGIN	33 (42)
ESCC-m	6 (8)
Mean length of USL, cm (± SD)	6.1 ± 2.9
Mean length of TA, cm (± SD)	8.1 ± 2.8
Pink-color sign at baseline	11 (14)
Baseline RFA regimen*	
A (Lugol-RFA-clean-RFA)	31 (40)
B (no Lugol-RFA)	24 (31)
C (Lugol-RFA)	14 (18)
D (Lugol-RFA-RFA)	8 (10)
Protocol follow-up phase (12-60 mo)	
Median follow-up, mo (IQR)	48 (48-48)
Median endoscopies per patient (IQR)	5 (4-6)
Patients with re-RFA	25 (37)
Total re-RFA sessions	59
Median re-RFA sessions per patient (IQR)	1 (1-1)

Values are n (%) unless otherwise defined.
ESCC-m, Mucosal esophageal squamous cell carcinoma; *HGIN,* high-grade intraepithelial neoplasia; *IQR,* interquartile range; *MGIN,* moderate-grade intraepithelial neoplasia; *RFA,* radiofrequency ablation therapy; *re-RFA,* reiterative RFA during the follow-up period; *SD,* standard deviation; *USL,* unstained lesion.
*Four different circumferential RFA regimens were used at baseline, A (Lugol-RFA-cleaning-RFA), B (no Lugol-RFA), C (Lugol-RFA), D (Lugol-RFA-no cleaning-RFA). One patient was treated with a different baseline regimen.[10]

scheduled within 3 (± 1) months. Finally, in 4 patients (4%) follow-up after treatment for recurrent ESCN was not performed according to protocol. These patients were not seen 3 (± 1) months after retreatment (PV-3), yet all 4 patients did return for the next annual follow-up endoscopy.

Sustained CR, recurrence, and progression

Of the 78 patients with CR12, 67 patients (86%) had sustained CR during follow-up (Fig. 2). In 25 of these 67 patients (37%), focal re-RFA sessions were performed for USLs that had no ESCN (ie, a USL was biopsy sampled and ablated in the same session, and subsequent histology showed no ESCN), with a median of 1 treatment session per patient (IQR, 1-1).

Of the 78 patients with CR12, 11 patients (14%) developed recurrent (n = 7) or progressive (n = 4) disease during follow-up (Fig. 2). The mean recurrence-free survival time was 45 months (95% confidence interval [CI], 43-47) (Fig. 3).

Seven patients (9%) had recurrent disease (flat MGIN [n = 6] or flat HGIN [n = 1]) (Fig. 4). Baseline pathology for these patients was MGIN (n = 4) or HGIN (n = 3). All recurrences were treated with re-RFA, with CR re-established in 4 patients and CR results pending in 3 patients who were treated at their last follow-up endoscopy.

Progressive disease was found in 4 other patients (5%), all with HGIN at baseline (Table 3). All were treated with ESD; 2 were diagnosed with disease stages that required additional nonendoscopic treatment, but both refused further therapy. Patient 1 presented with a flat USL containing MGIN 2 years after baseline and was treated with focal re-RFA. The patient returned 1 year later (PV-3) when a Paris type 0-IIa/c lesion was found. ESD was performed, and pathology showed poorly differentiated submucosal ESCC (ESCC-sm), invading the upper two-thirds of the submucosa. Patient 2 maintained CR through the 2-year follow-up endoscopy, but then after a 6-month prolonged follow-up interval (PV-1), a slightly elevated lesion was observed that was normally stained after Lugol's staining. ESD found an ESCC-sm, invading more than two-thirds of the submucosa that was buried under non-neoplastic epithelial cells (subepithelial disease) and had a positive deep resection margin. Patient 3 had sustained CR through his 2-year follow-up endoscopy but then did not return for 2 additional years (PV-1), at which time he was found to have a superficially elevated USL (Paris type 0-IIa). ESD showed a moderately differentiated ESCC-sm, invading the upper one-third of the submucosa. Patient 4 initially presented with persistent recurrent flat disease at 2, 3, and 4 years (HGIN, MGIN, and MGIN) and was repeatedly treated with re-RFA without achieving CR (PV-3). At the 5-year endoscopy a Paris type 0-IIa/c USL containing HGIN was found and resected with ESD.

Other secondary outcomes

There were no serious adverse events such as perforation, infection, bleeding requiring transfusion, or death during the study period. No new strictures were observed during the follow-up period. In the treatment phase a stricture occurred in 20 patients, as described previously.[11] Four of these patients required endoscopic dilatation (median, 1 session; IQR, 1-1) during this follow-up phase, and all strictures were resolved at the last study endoscopy. Stricturing during the treatment phase was not associated with recurrent or progressive disease during follow-up. Two patients developed a stricture after circumferential ESD, which was resolved after 7 and 8 dilatations, respectively.

ESCN-containing USLs outside the TA were found in 11 of 78 patients (14%; MGIN, n = 7; HGIN, n = 3; ESCC-m, n = 1). They were treated with RFA (n = 6) or ESD (n = 2) or a small USL that was biopsy sampled away and no ESCN was found during subsequent follow-up endoscopies (n = 3).

Clinical course of treatment failures at 12 months

Of the 96 patients initially included in the original study, the 12 with residual ESCN at 12 months were identified as

TABLE 2. Protocol violations

	Number of patients (%)	Median frequency of occurrence per patient (range)
Prolonged follow-up intervals (PV-1)	24 (31)	1 (1-3)
USLs that were left untreated (PV-2)	30 (38)	1 (1-4)
Inadequate follow-up after new ESCN (PV-3)	4 (5)	1 (1-3)
Any violation	46 (59)	1 (1-4)

Three main type of protocol violations occurred during the follow-up period; (1) patients with prolonged follow-up intervals (PV-1); (2) patients with USLs that were left untreated (PV-2); and (3) patients with inadequate follow-up after retreatment for recurrent ESCN (PV-3). Each PV could occur multiple times in a single patient.
ESCN, Esophageal squamous cell neoplasia; *PV*, Protocol violation; *USL*, unstained lesion.

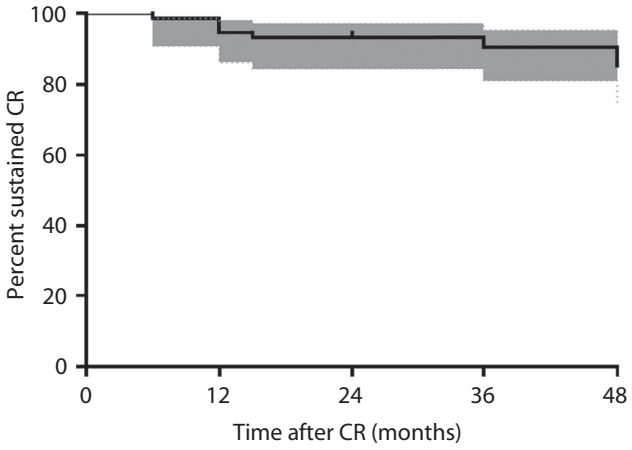

Figure 3. Kaplan-Meier curve for durability of ESCN eradication after initial successful RFA. *Black curve* (*gray zone* representing 95% confidence interval) shows the durability of CR12 after RFA for MGIN, HGIN, and early ESCN during the extended follow-up. Eleven patients with recurrent or progressive disease were considered to be failures, even if CR was re-established after reiterative radiofrequency ablation. *CR*, Complete response; *CR12*, CR at 12 months; *ESCN*, esophageal squamous cell neoplasia; *HGIN*, high-grade intraepithelial neoplasia; *MGIN*, moderate-grade intraepithelial neoplasia; *RFA*, radiofrequency ablation.

treatment failures (Fig. 2). These patients were treated and followed per investigator's discretion outside this formal follow-up study. We report on the clinical course after 12 months, realizing that both treatments and follow-up protocols varied in these patients and were not the same as those who achieved CR12.

Five (42%) of the 12 treatment failures achieved and sustained CR after additional RFA (ie, RFA sessions after the 12 months of the original study) (n = 4) or after EMR for a flat lesion containing HGIN (n = 1), and 1 was lost to follow-up directly after 12 months. The other 6 patients (50%) showed progressive disease and were treated with surgery (n = 4), chemoradiotherapy (n = 1), or ESD (n = 1) (Table 4). Three of these progressors had first achieved CR upon additional RFA and subsequently developed progressive disease, whereas the other 3 developed progressive disease from persistent ESCN. Of the 6 patients with progressive disease, 4 (67%) developed advanced ESCC that exceeded the limits of endoscopic therapy (1 ESCC-T3N1Mx, 1 ESCC-T1smN1M1, 1 ESCC-T2N0Mx, and 1 ESCC-T1smNxMx). One patient developed subepithelial disease that was not clearly visible on Lugol's chromoendoscopy (Table 4, patient 2; Fig. 5). This patient had a persistent USL with HGIN at 12 months and was treated with RFA twice, after which the USL had disappeared, and biopsy specimens were negative at 24 months. However, a small, atypical nodule had developed, with normal staining characteristics after Lugol's chromoendoscopy and with negative biopsy specimens. This lesion persisted on subsequent follow-up endoscopies, with repeatedly normal Lugol's staining and negative biopsy samples. Biopsy samples at the 60-month endoscopy showed ESCC, and the patient was referred for surgery. Pathology assessment showed a poorly differentiated ESCC-sm covered by non-neoplastic squamous epithelium. One month after surgery, an enlarged cervical lymph node and squamous gastric metastasis were detected.

Predictors for recurrence or progression

Of the 78 patients in CR12, 67 sustained CR, whereas 11 developed recurrent or progressive disease. On univariate analysis, the likelihood of developing recurrent or progressive disease during follow-up after RFA was associated with a longer baseline USL length (hazard ratio [HR], 1.26; 95% CI, 1.07-1.49) (Table 5). Multivariate testing including baseline USL length and PCS showed independent association with recurrent or progressive disease only for baseline USL length.

To identify predictors for failure to achieve or sustain CR during 5 years after initial RFA, additional analyses were performed that included all 90 patients (78 with CR12 + 12 treatment failures) and considered both treatment failures at 12 months and CR12 patients who developed recurrent or progressive disease during follow-up as failures. On univariate analysis, baseline USL length (HR, 1.22; 95% CI, 1.08-1.37) and PCS (HR, 4.01; 95% CI, 1.75-9.23) were significantly associated with failure after RFA (Supplementary Table 2, available at www.giejournal.org). Multivariate testing demonstrated that both USL length (HR, 1.20; 95% CI, 1.07-1.35) and PCS (HR, 3.66; 95% CI, 1.59-8.41) were independent predictors for failure to achieve or sustain CR during 5 years after initial RFA (Supplementary Fig. 1, available at www.giejournal.org).

DISCUSSION

This is the first study to assess the long-term durability of RFA treatment of MGIN, HGIN, and ESCC-m. In the 78

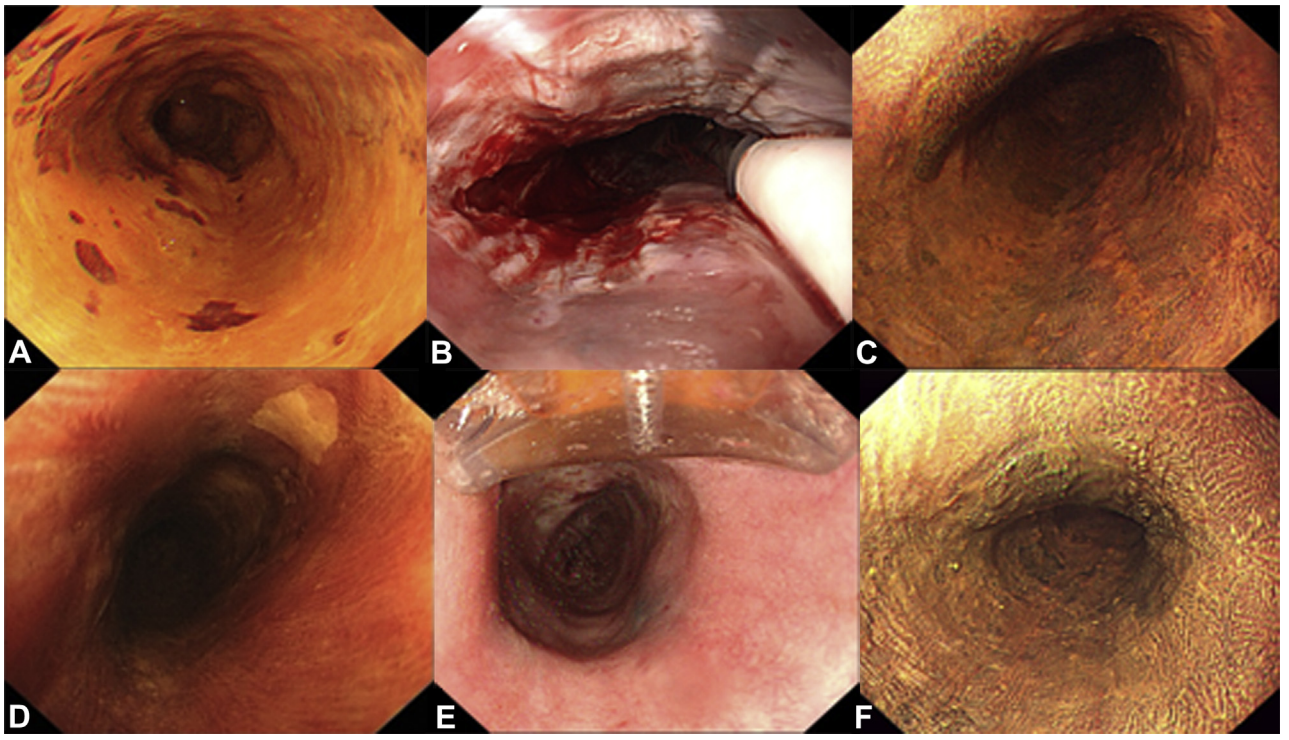

Figure 4. A recurrent USL during follow-up was successfully retreated with RFA. At baseline, this patient had a large USL that extended from 3 to 11 o'clock (**A**) with HGIN histology, which was treated with circumferential RFA (**B**). At 12 months, this patient was in complete remission with absence of USLs after Lugol's staining (**C**) and negative biopsy samples. One year later, a recurrent USL was found (**D**), with MGIN histology. The patient was re-treated with a single session of focal RFA (**E**), and during subsequent follow-up endoscopies through 60 months, the patient reachieved and sustained a complete remission (**F**). *HGIN*, High-grade intraepithelial neoplasia; *MGIN*, moderate-grade intraepithelial neoplasia; *RFA*, radiofrequency ablation; *USL*, unstained lesion.

patients whose RFA resulted in complete eradication of ESCN during the treatment phase (CR12), most (86%) sustained this eradication through 5 years after initial treatment. A few patients (9%) developed recurrent disease, and all could be treated with re-RFA. Progressive disease was observed in 5%, and half of these could be curatively treated with ESD. In contrast, the 12 patients whose ESCN eradication was not successful during the 12-month treatment phase had relatively poor outcomes. Although 42% achieved and sustained CR after additional RFA, 50% developed progressive disease, and most of these required nonendoscopic therapy. Two patients, including 1 with and 1 without CR12, developed subepithelial disease that was not clearly visible on Lugol's chromoendoscopy.

The results of this study are the only information currently available on the long-term outcome after RFA for ESCN. It is important to note that the study was limited by suboptimal follow-up and PVs in a substantial number of patients. Nevertheless, 3 important lessons can be learned from our data. First, cases in whom CR of ESCN is not accomplished within a 12-month treatment period are prone to disease progression. Most progressors and the most advanced progressors were found in the group of patients with persistent ESCN at 12 months. These patients should therefore undergo escape treatment by EMR or ESD at 12 months to allow full histologic review of the lesion and further treatment if clinically indicated. Second, RFA of ESCN requires rigorous endoscopic follow-up with Lugol's chromoendoscopy. Stringent follow-up is required, given the rate of recurrent lesions in the TA and the occurrence of metachronous lesions in the remaining esophagus. All USLs seen after Lugol's staining should be biopsy sampled for histologic diagnosis. Absence of USLs does not exclude the possibility of subepithelial neoplasia, and therefore all nonflat areas (ie, all lesions other than Paris type 0-IIb) in the TA should be sampled with keyhole biopsy samples, with a low threshold for a diagnostic EMR or ESD. Third, RFA should be restricted to patients with flat type (ie, Paris type 0-IIb) USLs that contain MGIN or HGIN and do not have a PCS after Lugol's staining. "Flat lesions" was already a major selection criterion in the current study; however, the distinction between 0-IIb and 0-IIa or 0-IIc may be difficult, especially in the West where ESCN is a rare disease and where endoscopists are less experienced with ESCN.[14] We suggest adding the PCS after Lugol's chromoendoscopy as an additional endoscopic exclusion criterion for RFA treatment. We believe that RFA should be restricted to patients with lesions limited to the epithelium (ie, MGIN or HGIN). The ablation effect of RFA covers the epithelium but may be insufficient for eradication of invasive neoplasia (ie, ESCC).[15-17] The

TABLE 3. Patients with progressive disease after an initial complete remission at 12 months

Patient no.	Baseline*	Time (mo)	Endoscopic appearance†	Escape treatment	Worst pathology‡
1	4-cm USL, HGIN, PCS+, regimen A	40	0-IIa, USL, 1 cm	ESD	ESCC-sm2, G3, LVI-, R0
2	11-cm USL, HGIN, PCS, regimen C	48	0-IIa, normally stained, 6 cm	ESD	Subepithelial ESCC-sm3, G2, LVI-, R1
3	10-cm USL, HGIN, PCS–, regimen A	48	0-IIa, USL, 1 cm	ESD	ESCC-sm1, G2, LVI-, R0
4	11-cm USL, HGIN, PCS–, regimen C	60	0-IIa/c, USL, 3 cm	ESD	HGIN

Progressive disease was defined as any nonflat lesion or detection of a more severe histologic grade than the grade at study entry.
ESCC-sm, Submucosal esophageal squamous cell carcinoma; *ESCC-sm1,* ESCC-sm invading the upper one third of the submucosa; *ESCC-sm2,* ESCC-sm invading the upper two thirds of the submucosa; *ESCC-sm3,* ESCC-sm invading more than two thirds of the submucosa; *ESCN, esophageal squamous cell neoplasia; ESD,* endoscopic submucosal dissection; *HGIN,* high-grade intraepithelial neoplasia; *LVI,* lymphovascular infiltration; *PCS+,* pink-color sign present; *PCS–,* pink-color sign absent; *R0,* radical resection with deep margins free of ESCN/ESCC; *R1,* radical resection with deep resection margin involved by ESCN/ESCC; *USL,* unstained lesion.
*Four different circumferential RFA regimens were used at baseline (see Table 1): A (Lugol-RFA-cleaning-RFA), B (no Lugol-RFA), C (Lugol-RFA), D (Lugol-RFA-no cleaning-RFA). One patient was treated with a different baseline regimen.[10]
†Appearance according to the Paris classification of endoscopic lesions[27]: 0-IIa = slight elevation of the mucosa, 0-IIb = flat mucosa, 0-IIc = slight mucosal depression, 0-IIa/c = combined type 0-IIa and 0-IIc lesion; the characteristics after Lugol's staining; and the maximum length in cm.
‡Differentiation according to American Joint Committee on Cancer[28]: G1 = well differentiated, G2 = moderately differentiated, G3 = poorly differentiated, G4 = undifferentiated.

TABLE 4. Patients with persisting esophageal squamous cell neoplasia at 12 months that subsequently developed progressive disease

Patient no.	Baseline*	12-Month PA	Moment of Progression (mo)	Endoscopic appearance†	Escape treatment	Worst pathology‡
1	6-cm USL, MGIN, PCS+, regimen A	HGIN	48	0-IIa/c, USL, 5cm	Surgery, chemotherapy	ESCC-T3N1Mx, G2, LVI-, R0
2	4-cm USL, ESCC, PCS+, regimen B	HGIN	60	Nodule, normally stained, .8 cm	Surgery, chemoradiotherapy	ESCC- T1smN1M1, G3, LVI+, R0
3	12-cm USL, HGIN, PCS–, regimen B	HGIN	42	0-I, USL	Surgery, chemotherapy	ESCC-T2N0Mx, G3, LVI-, R0
4	10-cm USL, HGIN, PCS+, regimen C	MGIN	55	0-IIa/c, USL, 6cm	ESD	ESCC-T1sm2NxMx, G2, LVI-, R0
5	6-cm USL, HGIN, PCS–, regimen B	HGIN	60	0-I, USL	Chemoradiotherapy	HGIN (biopsy diagnosis)
6	8-cm USL, HGIN, PCS–, regimen C	HGIN	36	0-IIa/c, USL, 2 cm	Surgery	HGIN, R0

Progressive disease was defined as any nonflat lesion or detection of a more severe histological grade than the grade at study entry.
ESCC, Esophageal squamous cell carcinoma; *ESD,* endoscopic submucosal dissection; *HGIN,* high-grade intraepithelial neoplasia; *LVI-,* no lymphovascular infiltration; *MGIN,* moderate-grade intraepithelial neoplasia; *PA,* pathology; *PCS+,* pink-color sign present; *PCS–,* pink-color sign absent; *R0,* radical resection with vertical margins free of ESCN/ESCC; *R1,* radical resection with positive vertical resection margin; *USL,* unstained lesion.
*Four different circumferential RFA regimens were used at baseline (see Table 1): A (Lugol-RFA-cleaning-RFA), B (no Lugol-RFA), C (Lugol-RFA), D (Lugol-RFA-no cleaning-RFA). One patient was treated with a different baseline regimen.[10]
†Appearance according to the Paris classification[27] of endoscopic lesion: 0-IIa = slight elevation of the mucosa, 0-IIb = flat mucosa, 0-IIc = slight mucosal depression, 0-IIa/c = combined type 0-IIa and 0-IIc lesion; the characteristics after Lugol's staining; and the maximum length in cm.
‡Differentiation according to American Joint Committee on Cancer[28]: G1 = well differentiated, G2 = moderately differentiated, G3 = poorly differentiated, G4 = undifferentiated.

pretreatment distinction between these 2 entities is therefore crucial, because RFA therapy lacks the histologic staging of ER. In our study the differentiation between ESCN and ESCC was based on endoscopic appearance (ie, type 0-IIb lesions only) and pretreatment biopsy specimens (with an inevitable risk of biopsy sampling error). We believe these 2 features may not suffice for adequate case selection before RFA. Therefore, we suggest adding the PCS as a third selection criterion. This reddish or rose-pink color change that typically occurs 2 to 3 minutes after Lugol's staining appears to be a characteristic of ESCC and more advanced stages of ESCN.[12,18-21] Although the recognition of the PCS seems rather straightforward and does not require advanced endoscopic skills, the inter- and intraobserver agreement has never been studied. Our results showed that the PSC was significantly associated with a failure to achieve or sustain CR after RFA: It independently predicted initial failure at 12 months (data not shown) and predicted failure to achieve or sustain CR during 5 years after initial RFA. The PCS at baseline identified 58% of the treatment failures at 12 months and 27% of the CR12 patients with recurrent or progressive disease during follow-up. Thus, the PCS may well be an extra safety criterion, which can partially overcome the current limitations inherent in macroscopic assessment and biopsy sampling error of ESCN.

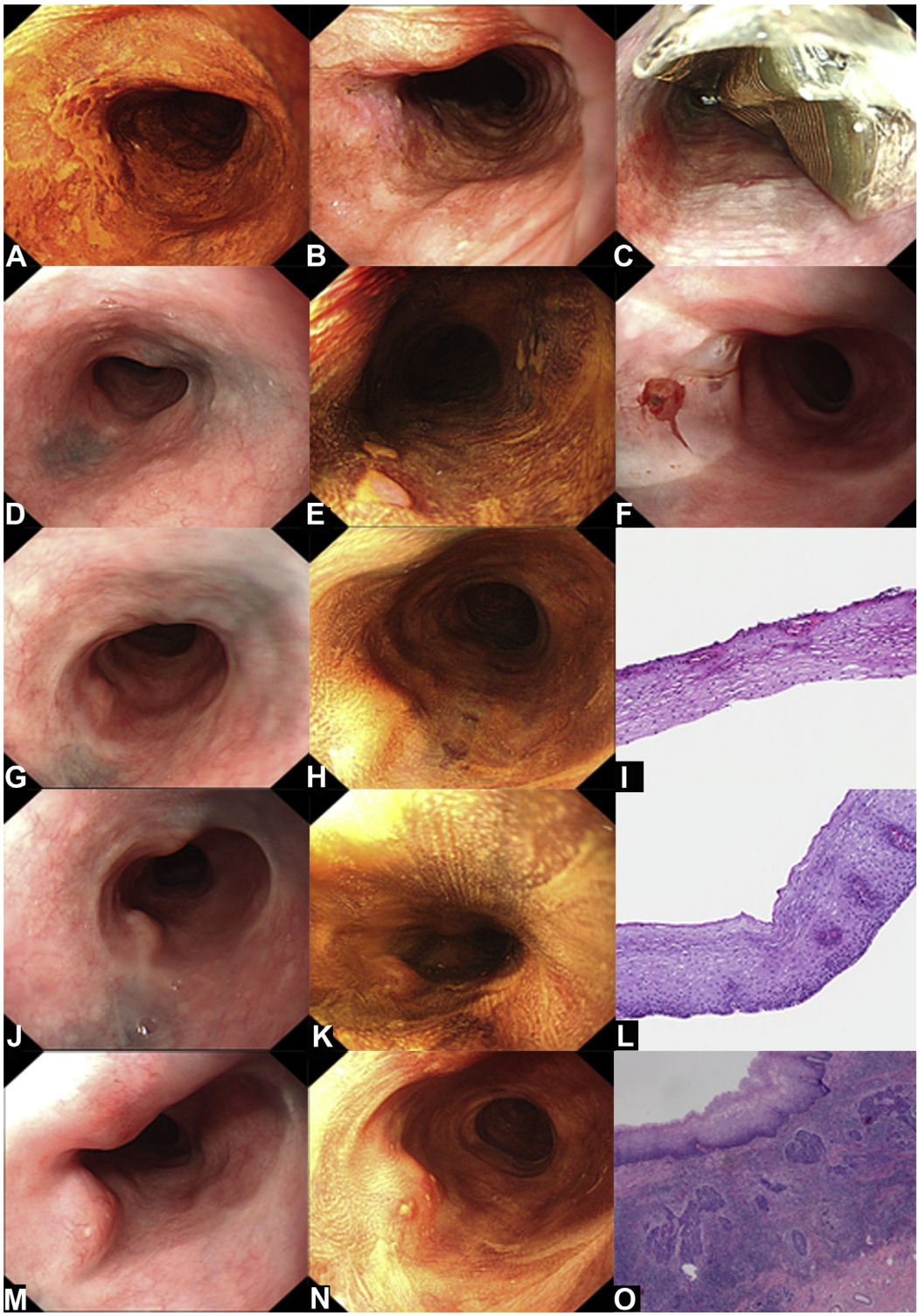

Figure 5. Post-RFA development of submucosal ESCC under normal squamous epithelium. Baseline images of the treatment area directly after Lugol's (**A**) and 2 to 3 minutes later, when a pink-color sign appeared (**B**). The patient was treated with 1 circumferential (**C**) and 2 focal RFA sessions in the treatment phase. At 12 months (**D**), the patient had a persisting USL with the pink-color sign (**E**) and HGIN in biopsy samples and was thus defined as a treatment failure. Treatment with focal RFA (**F**, after biopsy sampling and focal RFA) was continued for this USL at 15 and 18 months. At 24 months, a small nodule with normal overlying mucosa (**G**, *white arrow*) was seen, with normal staining after Lugol's (**H**), just distal from the 2 tattoos that were placed at the proximal margin of the initial treatment area. Endoscopic findings at 36 months were comparable (**J** and **K**). Targeted biopsy samples, obtained at 24 and 36 months, showed normal squamous epithelium (**I** and **L**). At 60 months the lesion appeared to have increased in size and now had a small erosion (**M**, *black arrow*). Lugol's staining was still normal, apart from the erosion (**N**). Targeted biopsy samples showed cancer, and surgery was performed. Histology showed a poorly differentiated submucosal ESCC under normal squamous epithelium (**O**). *ESCC*, Esophageal squamous cell carcinoma; *HGIN*, high-grade intraepithelial neoplasia; *RFA*, radiofrequency ablation; *USL*, unstained lesion.

TABLE 5. Predictors of recurrent and progressive disease during 5 years of follow-up

	Five-year follow-up		Cox analysis (univariate)	
	Sustained CR	Recurrence or progression	Hazard ratio [95% CI]	*P* value
All patients	67/78 (86)	11/78 (14)		
Baseline findings and treatments				
Baseline grade of ESCN				.40
MGIN	35/67 (52)	4/11 (36)	REF	REF
HGIN	26/67 (39)	7/11 (64)	2.33 [.68-7.98]	.18
ESCC-m	6/67 (9)	0/11 (0)	N.A.	N.A.
Median length of USL, cm (IQR)	5 (4-7)	10 (4-12)	1.26 [1.07-1.49]	<.01
Pink-color sign at baseline	8/67 (120)	3/11 (27)	2.64 [.70-9.96]	.23
Baseline RFA regimen*				.64
A	26/67 (39)	5/11 (45)	REF	REF
B	23/67 (34)	1/11 (9)	.27 [.03-2.33]	.24
C	11/67 (16)	3/11 (27)	1.53 [.37-6.41]	.56
D	6/67 (9)	2/11 (18)	1.52 [.30-7.85]	.62
Findings during the treatment or follow-up phase				
Post-RFA stricture	16/67 (24)	3/11 (27)	.96 [.25-3.60]	.95
LGIN at 12 mo	11/67 (16)	3/11 (27)	1.67 [.44-6.30]	.45
Protocol violations	37/67 (55)	9/11 (82)	2.53 [.55-11.70]	.24

Values are n (%) unless otherwise defined.
CI, confidence interval; *CR,* complete remission, defined as absence of MGIN or worse in pathology assessment; *ESCC-m,* mucosal esophageal squamous cell carcinoma; *ESCN,* esophageal squamous cell neoplasia; *HGIN,* high-grade intraepithelial neoplasia; *IQR,* interquartile range; *LGIN,* low-grade intraepithelial neoplasia; *MGIN,* moderate-grade intraepithelial neoplasia; *N.A.,* not applicable; *REF,* reference; *RFA,* radiofrequency ablation; *USL,* unstained lesion.
*Four different circumferential RFA regimens were used at baseline (see Table 1): A (Lugol-RFA-cleaning-RFA), B (no Lugol-RFA), C (Lugol-RFA), D (Lugol-RFA-no cleaning-RFA).[11]

Data on short-term efficacy and safety of RFA for treatment of ESCN are limited but promising.[10,11,22,23] In our original 12-month study, the currently advised RFA regimen (Lugol staining followed by a single 12 J/cm^2 ablation) was associated with CR12 in 82% and a stenosis rate of 6%.[11] This approach emerged as the optimal regimen (ie, comparable efficacy but a significantly lower stricture rate) from a number of small patient groups in which different regimens were used.[10,11] It is important to note that the number of cases treated with this "Lugol-12J" regimen was limited (only 17 cases), and these cases were assigned to this treatment on a temporal basis. In the current follow-up study, no significant differences were found for the 4 regimens in terms of durability, yet this analysis was likely underpowered. Recurrent disease did occur after all 4 regimens, and we believe there is no indication to change the initial advice for the optimal regimen. To evaluate this regimen further, a new prospective study using this regimen in 100 additional patients is underway that incorporates lessons learned from the current study (Table 6). Inclusion criteria for this new trial require patients to have flat type USLs with MGIN or HGIN and no PCS, and a more rigorous follow-up protocol will include standard follow-up endoscopies every 3 months in the first 12-month treatment phase independent of pathology results. Patients with ESCN persisting at 12 months (ie, treatment failures) will undergo EMR or ESD, whereas patients with a CR12 will enter a 4-year follow-up phase consisting of annual Lugol's chromoendoscopy with dedicated inspection by experienced endoscopists and histologic assessment of biopsy specimens. All lesions that exceed the initial inclusion criteria for RFA (ie, nonflat lesions, PCS-positive lesions, or lesions with an ESCC diagnosis) will be directly treated with EMR or ESD to prevent progression to advanced disease, and keyhole biopsy sampling will be performed on normal-staining non-flat lesions to evaluate the possibility of subepithelial ESCC. Based on this, we suggest 12 "do's and don'ts" to optimize the chance of a successful outcome for RFA treatment of ESCN (Table 6).

We found 2 patients with subepithelial ESCC that was not clearly visible after Lugol's staining. These patients developed a nonflat lesion, whereas the epithelium appeared normal on white-light endoscopy, narrow-band imaging, and after Lugol's staining. Pathology assessment for these patients showed ESCC-sm covered by non-neoplastic squamous epithelium. These subepithelial lesions are rightly feared for the risk they may pose to silently progress to advanced cancer without being visible on endoscopy. Besides being hard to detect, these lesions can be difficult to eradicate because it is easy to underestimate the true size of the tumor.[24] ESCN has the potential to extend down the pre-existing epithelial-lined ducts of submucosal glands. This neoplastic extension may go as deep as the submucosal layer, without the epithelial neoplasia actually being invasive ("submucosally located intraepithelial neoplasia"). This was shown by Tajima et al[25] in a study

TABLE 6. Lessons learned from the current study to optimize outcomes of RFA for ESCN

1	Perform workup with high-definition white-light endoscopy and 1.25% Lugol's chromoendoscopy to a) Thoroughly assess the Paris type of USLs. b) Sample USLs (1 level of biopsy samples per 1 cm of USL, with the number of biopsy samples per level to be determined as follows: 1 biopsy sample if the lesion covers <25% of the circumference, and 2, 3, or 4 biopsy samples for USLs covering 25%-50%, 50%-75%, or 75%-100% respectively). For example, for a 3-cm-long USL covering 50% of the circumference, 3 levels of 2 biopsy samples each (6 biopsy samples) are required. c) Look for the presence of the pink-color sign.
2	Perform EUS and CT for HGIN to exclude ESCC and metastatic disease.
3	Only include cases with type 0-IIB lesions containing MGIN or HGIN and no pink-color sign.
4	Wait at least 2 weeks after the last endoscopy with Lugol's staining before performing endoscopic treatment (the caustic effect of recent previous Lugol's staining is associated with increased bleeding).
5	Reinspect the esophagus with high-definition endoscopy and Lugol's staining at the RFA session to confirm that you are still dealing with only type 0-IIB lesions without the pink-color sign.
6	The currently recommended RFA regimen is 1 × 12 J/cm^2 for circumferential RFA and 3 × 12 J/cm^2 (no cleaning) for focal RFA.
7	Tattoo the proximal and distal margins of the treatment area with 2-minute ≤.5-mL injections.
8	Repeat follow-up endoscopies with Lugol's staining and biopsy sampling and RFA of all USLs >5 mm every 3 months during the treatment phase (the first 12 months).
9	Perform EMR/ESD for all nonflat USLs, pink-color sign lesions, or USLs with ESCC in biopsy samples during the treatment phase.
10	After the 12 month endoscopy (the end of the treatment phase): a) Perform EMR/ESD for patients with persisting ESCN. b) Refer patients with CR (absence of ≥MGIN) to the follow-up phase consisting of annual endoscopies.
11	Obtain biopsy specimens of all USLs during follow-up and obtain keyhole specimens of all nonflat lesions in the treatment area, even if there is normal Lugol's staining, with a low threshold for EMR/ESD.
12	Perform direct EMR/ESD of (1) all nonflat USLs, (2) all lesions with a pink-color sign, and (3) all lesions with ESCC in the biopsy samples.
13	In case of retreatment with RFA, begin follow-up again as if it were the initial treatment (follow steps 8-12 above).

These suggestions are based on our experience and the current limited data. We would advise using RFA cautiously for ESCN, only by experienced endoscopists, and according to the rules presented here.
CR, Complete remission; *ESCC,* esophageal squamous cell carcinoma; *ESCN,* esophageal squamous cell neoplasia; *ESD,* endoscopic submucosal dissection; *HGIN,* high-grade intraepithelial neoplasia; *MGIN,* moderate-grade intraepithelial neoplasia; *RFA,* radiofrequency ablation; *USL,* unstained lesion.

of 83 surgically resected specimens with ESCC-m: Neoplastic extension in these ducts was found in 14% (11/83), of which 45% (5/11) showed submucosally located intraepithelial neoplasia. Jansen et al[14] reported even higher rates of neoplastic extension into the ducts in 65 ESCC ESD specimens: Neoplastic ductal extension was found in 60% (39/65), with submucosally located intraepithelial neoplasia in 33% (13/39).

Extension of ESCN down pre-existing ducts of submucosal glands may be associated with residual ESCN after RFA treatment if the ablation remains too superficial. RFA intentionally aims to ablate the epithelium and muscularis mucosa but preserve the submucosa, reasoning that this will reduce the risk for adverse events like bleeding, fibrosis, and stricturing. Three studies have assessed the depth of ablation after RFA in humans at a histopathologic level. When different circumferential treatment regimens were used (either 1, 2, or 3 hits with 8-14 J/cm^2),[15-17] all reported damage limited to the mucosa without any injury in the submucosa. However, the depth of ablation in these studies was only assessed through 2 days after ablation, and ablation may extend deeper over subsequent days. An animal study that assessed ablation depth after RFA in pig esophagi reported more advanced injury at 3 days postablation than at 0, 2, 5, or 7 days postablation.[26] Further study with a larger number of patients and careful evaluation with follow-up endoscopies by experienced endoscopists will be required to further evaluate the risk of subepithelial ESCN after the use of RFA for squamous neoplasia.

This is the first long-term follow-up study after RFA for patients with ESCN. Strengths of our study include the prospective design, the relatively large number of patients, and that most patients completed 60 months of follow-up. Furthermore, we used a standardized biopsy sampling protocol with a large number of biopsy sampling performed to sample both the normally stained TA and recurrent or persistent USLs at each visit, and all biopsy specimens were reviewed by an expert GI pathologist with selected review by a second expert. Last, we used clear histologic endpoints.

Two major limitations need to be addressed. The first was the suboptimal follow-up with PVs in a substantial number of patients. Although no association was found between PVs and the occurrence of recurrent or progressive disease (Table 5), this still may have affected our results, and the results might have been better if we had strictly adhered to the study protocol. Although this would not have influenced the incidence of recurrent disease, it might have contributed to disease progression in some of our patients. This emphasizes the importance of strict follow-up regimens with a low threshold for escape

treatment during treatment and follow-up phases and the need for a new prospective study.

The second major limitation was the use of different RFA regimens at baseline. Given the relatively small numbers of patients per subgroup, the temporal assignment of cases to each subgroup and the variability of regimen among the subgroups, the long-term results by treatment group were of little value in the choice of an optimal treatment regimen. We believe our follow-up results indicate the need to be cautious with RFA for ESCN in general, without dose-specific recommendations.

Another limitation of our study was the post-hoc assessment of the PCS by reviewing pictures of the baseline endoscopy. The PCS was first reported in the literature as a predictor of HGIN or ESCC during the course of our study, and, therefore at baseline we did not report on its presence.

In conclusion, most patients (86%) with ESCN or ESCCm who had a CR12 (no residual disease) after initial RFA treatment experienced sustained eradication of neoplasia during an additional 4 years of follow-up. Among the few patients with a CR12 who experienced new ESCN during follow-up, most could be treated with RFA and only a few showed progression of disease. Patients with residual ESCN at 12 months, however, were at higher risk for progression to advanced disease. Overall, a significant number of those who progressed developed advanced ESCC that required non-endoscopic therapy, and 2 patients developed subepithelial ESCC that was not clearly visible on Lugol's chromoendoscopy.

Based on our study, which was limited by PVs and inadequate follow-up in a significant number of patients, we conclude that RFA may be best suited for treatment of noninvasive epithelial neoplasia (ie, MGIN or HGIN) and cannot be recommended for treatment of ESCC. Given the complexity of differentiating ESCN from ESCC before treatment, we advise the cautious use of RFA and only by endoscopists highly experienced in differentiating ESCN from ESCC. We have suggested several "lessons learned" (Table 6) to optimize the chance of a successful outcome. Additional studies with careful selection of patients and strict treatment and follow-up protocols performed by experienced endoscopists are ongoing and are needed to further clarify the role of RFA in the treatment of patients with ESCN.

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Abbreviations: CI, confidence interval; CR, complete remission; CR12, complete remission at 12 months; ESCC, esophageal squamous cell carcinoma; ESCC-m, mucosal esophageal squamous cell carcinoma (=ESCC-T1m); ESCC-sm, submucosal esophageal squamous cell carcinoma (=ESCC-T1sm); ESCC-T2, esophageal squamous cell carcinoma, invading the muscularis propria; ESCC-T3, esophageal squamous cell carcinoma, invading the adventitia; ER, endoscopic resection; ESCN, esophageal squamous cell neoplasia; ESD, endoscopic submucosal dissection; HGIN, high-grade intraepithelial neoplasia; HR, hazard ratio; IQR, interquartile range; MGIN, moderate-grade intraepithelial neoplasia; PCS, pink-color sign; RFA, radiofrequency ablation therapy; re-RFA, reiterative radiofrequency ablation therapy (ie, after 12 months); PV, protocol violation; PV-1, protocol violations type 1; PV-2, protocol violations type 2; PV-3, protocol violations type 3; TA, treatment area; USL, unstained lesion.

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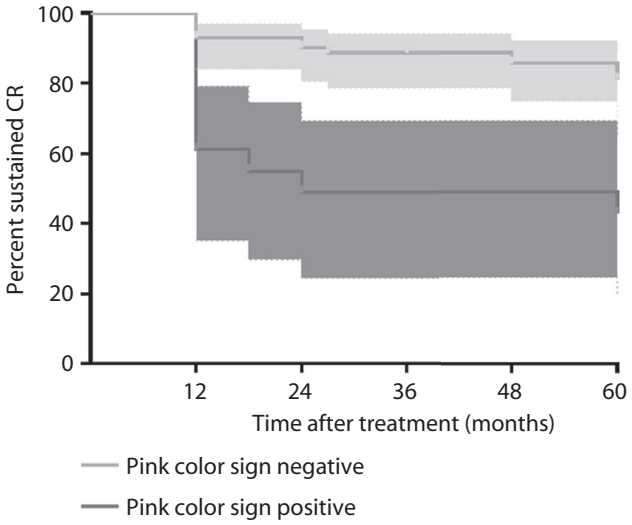

Supplementary Figure 1. Kaplan-Meier curve showing the durability of radiofrequency ablation, stratified for pink-color sign at baseline. *Dark curves* (*light zones* representing 95% confidence interval) show the durability of complete remission after radiofrequency ablation for MGIN, HGIN, and early ESCN during 5 years of follow-up. Patients with persistent ESCN at 12 months and patients with initial CR that developed recurrent or progressive disease after 12 months were considered to be failures, even if CR was re-established after reiterative radiofrequency ablation. *CR,* Complete remission; *ESCN,* esophageal squamous cell neoplasia; *HGIN*, high-grade intraepithelial neoplasia; *MGIN*, moderate-grade intraepithelial neoplasia.

SUPPLEMENTARY TABLE 1. Circumferential RFA regimens used at baseline in the initial study

Group	Regimen	Reasoning	No, of patients*	CR12 n (%)	Stricture n (%)
A	Lugol's RFA (12 J/cm^2) Clean RFA (10 or 12 J/cm^2)	Standard treatment regimen used for treatment of Barrett's esophagus	34	31 (91)	5 (15)
B	No Lugol's RFA (10 or 12 J/cm^2) No clean RFA (10 or 12 J/cm^2)	Lugol's solution might augment an inflammatory response, causing a higher stricture rate after RFA compared with BE patients (no Lugol's is used in BE)	28	24 (86)	13 (46)
C	Lugol's RFA (12 J/cm^2) No clean	Lugol's was reintroduced and the second ablation was eliminated to reduce thermal injury	17	14 (82)	1 (6)
D	Lugol's RFA (10 or 12 J/cm^2) No clean RFA (10 or 12 J/cm^2)	Because of the low stenosis rate in the previous regimen, it felt safe to reintroduce a second RFA application	10	8 (80)	1 (10)

BE, Barrett's esophagus; *CR*, complete remission, defined as complete eradication of moderate grade intra-epithelial neoplasia or worse; *CR12*, CR at 12 months; *RFA*, radiofrequency ablation.
*One patient was treated with a different regimen, consisting of 2 hits with 12 J/cm^2, with cleaning in between and without Lugol's staining. This patient did not develop a stricture and had CR12.

SUPPLEMENTARY TABLE 2. Predictors for failure after RFA in the total study population initially included in the primary study

	Five-year study		Cox analysis (univariate)	
	CR12 with sustained CR	Failures (at 12 mo or during follow-up)	Hazard ratio [95% CI]	*P* value
All patients	67/90 (74)	23/90 (26)		
Baseline grade of ESCN				.06
MGIN	35/67 (52)	5/23 (22)	REF	REF
HGIN	26/67 (39)	15/23 (65)	3.42 [1.24-9.44]	.02
ESCC-m	6/67 (9)	3/23 (13)	3.18 [.76-13.33]	.11
Median length of unstained lesions, cm (IQR)	5 (4-7)	8 (5-11)	1.22 [1.08-1.37]	<.01
Pink-color sign at baseline	8/67 (12)	10/23 (43)	4.01 [1.75-9.23]	<.01
Baseline RFA regimen*				.66
A	26/67 (39)	8/23 (35)	REF	REF
B	23/67 (34)	5/23 (22)	.80 [.26-2.46]	.70
C	11/67 (16)	6/23 (26)	1.68 [.58-4.86]	.34
D	6/67 (9)	4/23 (17)	1.80 [.54-5.97]	.34
Stricture after initial RFA	16/67 (24)	4/23 (17)	1.52 [.52-4.47]	.45

Values are n/N (%) unless otherwise defined. Patients that failed to achieve CR12 (n = 12) and patients with CR12 that developed recurrent or progressive disease during follow-up (n = 11) were defined as failures.
CI, Confidence interval; *CR*, complete remission, defined as absence of MGIN or worse in pathology assessment; *CR12*, CR at 12 months; *ESCC-m*, mucosal esophageal squamous cell carcinoma; *ESCN*, esophageal squamous cell neoplasia; *HGIN*, high-grade intraepithelial neoplasia; *IQR*, interquartile range; *MGIN*, moderate-grade intraepithelial neoplasia, *REF*, reference; *RFA*, radiofrequency ablation; *USL*, unstained lesion.
*Four different circumferential RFA regimens were used at baseline (see Table 1): A (Lugol-RFA-cleaning-RFA), B (no Lugol-RFA), C (Lugol-RFA), D (Lugol-RFA-no cleaning-RFA).[11]