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SIGNIFICANCE OF NISSEN FUNDOPLICATION AFTER RADIOFREQUENCY ABLATION FOR PATIENTS WITH BARRETT'S ESOPHAGUS

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ZNAČAJ KOMBINOVANE RADIOFREKVENTNE ABLACIJE I ANTIREFLUKSNE HIRURŠKE PROCEDURE U LEČENJU BOLESNIKA SA BARRETT-OVIM JEDNJAKOM

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Dedicated to my parents

Significance of Nissen fundoplication after radiofrequency ablation for patients with Barrett's esophagus

Abstract

Barrett's esophagus (BE) is a metaplastic condition where normal squamous epithelium of the esophagus is replaced with columnar epithelium which contains goblet cells. Radiofrequency endoscopic ablation (RFA) of BE is a relatively new procedure, with already proven safety and efficacy profile, in the complete eradication of BE (CR-IM). The aim of this study was to present our data regarding the utilization of RFA in the treatment of metaplastic and dysplastic BE. The basic goal was to evaluate the role of post RFA treatment in the term of BE recurrence prevention. We also evaluated morphologic structure of neosquamous epithelium (NSE) obtained from biopsy samples and processed on electron microscopy.

The data indicated that in patients with long segment BE and hiatal hernia>3 cm, post RFA Nissen fundoplication has better protective effect than medical treatment. The morphologic structure of NSE showed presence of dilated intercellular spaces, which were present in higher extent in those patients who were treated with medications.

To conclude, RFA procedure is safe and effective in the treatment of patients with BE. In selected patients antireflux surgery may be offered as a standard treatment due to its protective nature over NSE.

Key words: Barrett's esophagus, radiofrequency ablation, Nissen fundoplication, proton pump inhibitors

The field of scientific interest: Digestive diseases, Barrett's esophagus

Značaj kombinovane radiofrekventne ablacije i antirefluksne hirurške procedure u lečenju bolesnika sa Barrett-ovim jednjakom

Abstrakt

Barrett-ov jednjak (BJ) predstavlja metaplaziju i nastaje kada se normalni skvamocelularni epitel jednjaka transformiše u cilindrični epitel koji sadrži peharaste ćelije. Radiofrekventna endoskopska ablacija (RFA) je relativno nova procedura čija je bezbednost i efikasnost već dokazana u kompletnoj eradikaciji Barrett-ovog jednjaka. Cilj ove studije bio je da se prikažu naši podaci u lečenju BJ radiofrekventnom ablacijom jednjaka, a osnovi cilj studije bio je da se proceni značaj primenjenog tretmana nakon RFA u smislu prevencije recidiva BJ. Takođe, učinjena je evaluacija morfološke strukture neoskvamoznog epitela na osnovu endoskopskih biopsija koje su obrađene i pregledane elektronskom mikroskopijom.

Naši podaci su ukazali da bolesnici koji imaju duži segment BJ i hijatalnu herniju >3 cm imaju manji stepen recidiva ukoliko je laparoskopska Nissen-ova fundoplikacija načinjena nakon RFA u odnosu na one koji su dobijali medikamentoznu terapiju. Analiza morfološke strukture neoskvamoznog epitela ukazala je da u ovom epitelu postoje dilatirani međućelijski prostori , ali da su u manjoj meri izraženi kod bolesnika koji su nakon RFA operisani.

U zaključku, RFA je sigurna i efikasna procedura u lečenju bolesnika sa BJ. U određenoj grupi bolesnika antirefluksna hirurgija ima prednost kao standardni tretman nakon uspešne ablacije u cilju prevecije recidiva oboljenja u odnosu na medikamentoznu terapiju.

Ključne reči: Barrett-ov jednjak, radiofrekventna ablacija, Nissen-ova fundoplikacija, inhibitori protonske pumpe

Naučna oblast: Digestivne bolesti, Barrett-ov jednjak

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1. Introduction

1.1. Definition

When Norman Barrett wrote his paper in 1950 he couldn't even imagine how often his name will be mentioned when talking about one of the most intriguing and unsolved clinical problems - Barrett's Esophagus (BE). Although, he reported it as a congenitally short esophagus, it was a first description of what we now know refer to as "presence of metaplastic or dysplastic columnar epithelium on the esophagus". [1] However, it was another great work by Allison and Johnston in 1953, in which first actual recognition of columnar lined esophagus was made. [2] In late seventies of past century Barrett esophagus is being recognized as a precursor of esophageal adenocarcinoma (EAC), and since than, incidence of EAC has increased dramatically, thus becoming the fastest growing incidence of all solid cancers in Western world. [3] It was this widespread of EAC that draw attention to the Barrett's esophagus, making it recognizable as a consequence of gastroesophageal reflux and a cause of carcinoma.

Today we now that Barrett's esophagus (BE) is result of long lasting gastroesophageal reflux (GER). Its nature is metaplasia, the process in which one adult cell replaces another, as a response to chronic tissue injury.[4] The prolonged exposure to the pathological reflux may bring the changes in the esophageal squamocellular epithelium as well as the growth of the new metaplastic columnar epithelium. The columnar epithelium formed in this manner should be more resistant to the damage caused by pathologic GER.

The most widely accepted definition in the medical community nowadays explains Barrett's esophagus is a 'metaplastic condition where the squamous esophageal epithelium is replaced by the columnar epithelium containing goblet cells'. [5]

Although this definition is accepted in majority of the countries globally, there are some of those who practice slightly different definition. For example in Japan and Great Britain every macroscopically visible epithelium, columnar in its nature, which extents more than 1 cm above the level of gastroesophageal junction is being considered as BE.[6] This standpoint may be more justified since it diminishes the probability of error, derived from the physician's personal experience and the quality of the biopsy sampling. However, the American Gastroenterological Association in 2011 made a clear statement, that the presence of goblet cells in columnar epithelium is essential for the diagnosis of BE. This standpoint is

explained by general opinion that only metaplastic epithelium containing goblet cells predisposes to adenocarcinoma. Opposed to this, several publications proved that adenocarcinoma of the esophagus can develop from columnar mucosa, which does not contain goblet cells. [7] These studies derived the conclusion that glandular mucosa on esophagus caries similar cancer risk as specialized intestinal metaplasia. It was also shown that DNA of glandular mucosa on tubular esophagus, which does not contain goblet cells, is similar to the one which does, and that this DNA profile is the one that caries risk for neoplastic progression.[8]

The dilemma about the BE definition is not the only one surrounding this clinical issue. The true incidence of BE, pathogenesis, surveillance and treatment issues are jet to be studied closely and standardized. Meanwhile, the emergence of HALO radiofrequency ablation for the treatment of metaplasitic and dysplastic BE has brought a new shift of the paradigm when it comes to BE management. [9] This study will address the efficacy of HALO RFA in the treatment of BE with the special emphasis on the post RFA treatment strategies.

1.2. Epidemiology of Barrett's Esophagus

The true incidence and prevalence of Barrett's esophagus remains unknown. This can be explained with the "quiet" symptomatology that BE caries and lack of objective data and large epidemiologic studies which would focus on detection of BE. These studies however, should cover broad population undergoing screening endoscopies, which makes them difficult to be well designed and conducted. Differences among different parts of the world in the prevalence of GERD, which is the major determinant of BE basis, make it more difficult for investigators to accurately determine its global prevalence.

Several epidemiologic studies, based on the national surveys, can give us data from which we can make further assumptions and draw an estimated prevalence of population affected with BE. Swedish epidemiologic study covered a random sample population, and 1000 participants in whom upper GI endoscopies were performed. The specialized intestinal metaplasia was proven in 16 participants (5 with long segment BE, 11 with short segment), with the conclusion that BE was found in 1.6% of general population in Sweden. [10] In the US based endoscopic

survey, 961 participants underwent upper GI endoscopy, during the scheduled colonoscopy. This study showed an overall prevalence of BE to be 6.8%, with majority of those found to have short segment BE. [11] Similar prevalence of BE in general population was found (5.6%) when simulation model was used by the US authors using the national data base. [12]

During the last two decades there has been a growing number of publications dealing with risk factors for the development of BE and EAC. Based on the current knowledge we can emphasize several factors that have been proven to contribute to the BE development.

- Demographic factors: White race, male sex (male to female ratio 2:1), and older age (>50 years) are risk factors for the development of BE and EAC. Male sex and white race contribute BE progression to EAC. [13]
- GERD: Gastroesophageal reflux disease is a well-known risk factor for both BE and EAC. It is estimated that around 12% of patients with diagnosed GERD will develop BE. The risk of BE development and further progression to EAC is also dependent on GERD symptom duration and intensity. [14]
- Smoking: There are strong evidences that smoking is associated with an increased risk for the BE and EAC. This factor has been proven to be dose-related and duration-related.
- Obesity: Obesity (but not BMI) is a strong risk factor for BE development. It appears that there is special role of visceral adiposity in BE and subsequent EAC development. There are two mechanisms behind pro-oncogenic role of visceral adiposity. First is increased intraabdominal and intragastric pressure, which correlates with the intensity of GERD, and the second is activation of metabolic syndrome.[15]
- There are evidences that Helicobacter pylori infection, and regular intake of NSAID/Aspirin are related to decreased risk of BE and EAC.

1.3. Pathogenesis

The basic process behind etiology of Barrett's esophagus is metaplasia. Metaplasia typically arises after prolonged tissue stress, or in response to abnormal tissue stimulation. [4] The nature of metaplastic epithelium should be defensive, meaning that this type of epithelium is more resistant to continuous

pathologic exposure. In the case of BE this prolonged tissue stress and stimulation is a consequence of chronic exposure of distal esophagus to gastric and duodenal contents. Patients with BE usually have defective function of lower esophageal sphincter (LES), resulting in unobstructed flow of gastric juice toward tubular esophagus. Among patients with GERD, those with BE are more often presented with low values of LES basal pressure, ineffective esophageal motility and completely displaced gastroesophageal junction (GEJ) due to hiatal hernia. These factors also contribute to the length of BE. It has been documented that patients with long segment BE have lower median values of LES basal pressure than those with short segment BE.[16]

The process of BE formation can be divided in two phases. The first step is probably development of the so-called cardiac mucosa, a simple columnar epithelium, which does not contain goblet cells, nor does it secret acid or pepsin. This concept, although not universally accepted suggest that cardiac mucosa is an acquired condition which develops as a consequence of reflux exposure.[17] As the reflux disease progresses, LES gradually losses its function, and the reflux episodes become more frequent and longer. This process may lead to the increase of the cardiac mucosa length. The second phase in the development of BE would be intestinalization of cardiac mucosa trough the development of goblet cells. The BE mucosa is called intestinal because all of the main mucosal intestinal cell types may develop inside this specialized epithelium. These are goblet cells, Paneth cells, enteroendocrine cells and enterocytes. [18]

The composition of refluxed content is one of the predisposing factors in the development of BE. In the first phase, which is the development of cardiac mucosa, there is probably a crucial role for acid reflux. Not only refluxed gastric acid but bile salts as well, have the decisive part in the process of intestinalization. There are two different suggested models how bile salts can contribute to BE formation. At the pH level 3-6 bile salts are soluble and non-ionized, and they affect cell junctions by exhibiting detergent like action. But what is more important in the pathogenesis of BE, bile salts may act as signaling molecules. It has been shown that in vitro culture of squamous cell epithelium (SCE) bile salts promote loss of differentiation of SCE. It is suggested that this activity is being conducted by activation of epidermal growth factor and down-stream of Akt and ERK1/2. There is a strong activity inside the injured epithelium towards damage repair, the

process which may end up in development of BE. [19]

Jet, another controversy is present in the pathogenesis of BE and that is the cell origin of BE. There are several hypotheses so far, but there are not enough evidences for none of those to be rejected or generally accepted. A recent hypothesis was that BE develop when cells from the level of the esophagogastric junction migrate upwards. This theory can explain the presence of fundic or gastric type columnar epithelium on tubular esophagus. [20] However, it can not explain the development of BE after esophagectomy and gastroplasty, in which case the GEJ region is resected. There is also a theory that BE develop from residual embriogenic type columnar cells, which rests there after the embriogenic development of esophagus and which are also located on the level of GEJ.[21] During the embriogenic development of esophagus these cells are being gradually "pushed down" by squamous cells. Another explanation of BE origin is that damaged matured SCE is going through the process of conversion to different epithelium type, a so called "transdifferentiation".[22] It is suggested that epithelial stem cells which are responsible for continuous reloading of SCE, under the reflux induced damage start to differentiate towards the columnar cell type. [23] The origin of these stem cells could be inside the basal layer of SCE or in the sub-mucosal esophageal glands. There is also a possibility that BE can derive from bone marrow progenitor circulating cells drawn there by chronic inflammation and strong molecular activity for mucosal repair as it has been shown in the rat model of reflux esophagitis.[24]

There is a hypothesis that there can be even more that one origin of BE cells, and that further progression of BE is determined by the origin cell type from which BE has developed.

1.4. Diagnosis and Classification

The diagnosis of Barrett's esophagus requires endoscopic visualization of columnar epithelium above the level of gastroesophageal junction lining the tubular esophagus, and esophageal biopsy histological confirmation of intestinal metaplasia. The physician performing upper GI endoscopy must evaluate several important landmarks, essential for the diagnosis and proper classification of BE. The gastroesophageal junction is verified as the top of the gastric folds. If the

metaplastic mucosa is present, it is identified as a “salmon” or pink colored with a rough surface, as opposed to squamocellular epithelium, which is pale gray and smooth. The squamocolumnar junction (SCJ) is than verified, and it is usually displaced proximally, and most often irregular due to the “tongues” of metaplastic epithelium extending upwards. [25] The sensitivity of white-light endoscopy alone for the detection and diagnosis of BE ranges from 80% to 90%. Novel endoscopes facilitates the imaging by employing several new technologies, such as chromoendoscopy, different magnification techniques, and confocal endoscopy, all of which contribute significantly to proper diagnosis of BE. These techniques also enables enchased visualization of suspicious dysplastic lesions inside BE. [26]

Traditional classification of BE as the long (>3cm) and short segment BE (≤3cm) still has its place in everyday clinical practice. It has been shown that patients with long segment BE have higher risk of progression to dysplasia and EAC. What remains more difficult for endoscopist is the identification of short segment BE. Again, this is where anatomical landmarks must be identified correctly, especially the top of the gastric folds. The identification of submucosal palisade vessels zone may be helpful, as these vessels are present on tubular esophagus, while columnar epithelium covering those could be with great certainty proclaimed as metaplastic and must be sampled for histologic evaluation. [27]

A traditional short and long segment grading segment is replaced with more standardized and validated grading protocol, the *Prague C&M classification* of BE. The C stands for circumferential extent of columnar epithelium in centimeters, and M for maximal extent of columnar epithelium in tubular esophagus. Again, the most important landmark is the top of the gastric folds, which must be properly determined as the measurement of C and M starts from that point. Several studies have validated the application of Prague C and M criteria on the expert level, where it has proved to have high interobserver agreement, but it has also gain wide acceptance and was validated in real time endoscopy and community hospitals.[28]

The histologic examination is based on esophageal biopsy sampling. The technique and principle of biopsy is extremely important. That is why standardized biopsy protocol was created (*The Seattle Protocol*). This protocol consists of four quadrant jumbo forceps biopsies on every 1 cm of BE with separate biopsies of eventual mucosal abnormalities. This rigorous protocol has been put to question

when Kariv et al proved that it does not more reliably predict the detection of cancer in patients with high grade dysplasia, than less invasive biopsy protocols. [29] Still, the following of defined protocol enables more trusting primary diagnosis and therefore a possibility od adequate surveillance or treatment. Method of taking the biopsy sample should be standardized as well. Experts recommend usage of "jumbo" forceps and a specific technique. Stomach must be desuflated during evaluation of GEJ, because overinsuflation can flatten the gastric folds. Than, the biopsy forceps jaws must be perpendicular to the surface of the tubular esophagus, and the suction must be applied to bring the esophageal mucosa as close as it can get to the top of the endoscope. This technique is being called "turn and suction"

The spectrum of histological findings inside BE is by convention divided into four basic groups. [30, 31]

1. *Columnar epithelium containing no dysplasia*
   - There are three distinct types of non-dysplastic columnar epithelium in the BE setting. Those are: gastric fundic type or oxintocardiac mucosa (containing glands, absence of goblet cells), cardiac type (columnar epithelium which contain no glands, and has marked inflammatory activity) and intestinal metaplasia (columnar epithelium which contain goblet cells). Only the later of three is proven to have malignant potential, and is being consider as the only histology type by AGA and American College of Gastroenterology (ACG) that can be proclaimed as Barrett's esophagus. Two different subtypes of intestinal metaplasia (IM) can be identified, "complete and incomplete IM". Incomplete IM have is unstable epithelium with higher risk of progression towards dysplasia and EAC.
2. *Indefinite for dysplasia*
   - This histologic type is utilized when there is uncertainty whether the epithelial changes are caused by cellular atipia or substantial inflammation. What characterizes this histologic category is a surface maturation, but the presence of irregular nuclei inside the deep glands.
3. *Low grade dysplasia (LGD)*
   - The crucial histologic finding in this BE subtype is cellular atipia which extends towards mucosal surface. The polarity of cell nucleus is preserved.

4. *High grade dysplasia (HGD)*
   - Histological findings inside HGD show both cytologic and architectural abnormalities including loss of nucleus polarity, possible presence of atypical mitoses and glandular distortion. Practically, HGD can be consider as carcinoma in situ, with malignant cells that do not invade lamina propria.

1.5. Natural Evolution of Barrett's Esophagus

When we use the term "natural evolution" of Barrett's esophagus we usually think about the progression from BE intestinal metaplasia to dysplasia and esophageal adenocarcinoma. It is estimated that patients with BE have 35- 125 times more chance to develop EAC than those without BE. Still, the great majority of people diagnosed with BE will never develop EAC.

Patients with BE intestinal metaplasia without dysplasia have low risk for developing EAC. A great number of studies had this issue examined over the past years. Well-designed and contemporary studies have shown that risk of progression from IM to EAC range from 0.12% to 0.43% per year. If we include high-grade dysplasia with EAC as a final endpoint of progression the risk rises to approximately 0.23- 0.63% per year. [32] Among those with non-dysplastic BE there is higher risk towards progression to EAC for patients with long segment BE. It is estimated that risk for progression increases significantly with every cm of BE length. [33] These data are not in concordance with previous studies, which showed that risk of progression for BE IM approximately 0.5%. These were the data from often-cited meta-analysis conducted by Shaheen at al., which took into account 25 articles dated from 1984 untill 1998. [34] What makes this study less objective for general population is that volume of the studies included in the meta-analysis directly influenced the results, in the manner that smaller studies reported higher risk of progression. The nationwide cohort study in Denmark conducted by Hvid Jansen et al reported however very low risk of annual progression to EAC among BE patients, which was shown to be 0.12%, and 0.26% if HGD was taken into account. [35] This study pointed that patients with IM BE have 11 times more chance of developing EAC compared to general population.

The risk of developing malignancy rises for those BE patients who develop low-

grade or high-grade dysplasia. A study by Reid and colleagues showed that 4% patients with BE LGD progress to EAC in a 5-year period, and that 61% of those with BE HGD would develop EAC in a 5-year period. [36] The differences were showed among patients regarding the speed of progression, where some patients have stable disease over time before rapidly progressing to EAC, while others progress slowly in steady pace. [37]

There are some issues that must be taken into consideration when we analyze the novel data concerning the rate of progression of non dysplastic BE to EAC and its role when deciding about conduction of the surveillance and treatment in this subset of patients. For example, for patients with non-dysplastic BE the leading gastroenterological associations worldwide do not recommend endoscopic treatment of BE, but rather advise different surveillance protocols. [5, 38] Still, ASGA guidelines recommend endoscopic radiofrequency ablation treatment of BE for patients with proven HGD, LGD, but also for selected cases on non dysplastic BE. [39] This is the reason why a physician must take into account the risk factors for progression such as duration of reflux symptoms, hiatal hernia size, length of BE segment, gender, ethnicity, obesity and others when considering the general risk of progression towards EAC.[40]

In an interesting paper, which was set to introduce the case for non-dysplastic, BE ablation, authors address several very important issues. [41] The first is that BE risk of progression towards EAC is artificially underestimated because of the current tendency not to count the patients with prevalent cancer and HGD occurring one year from diagnosis and the second is the problem of BE overdiagnosing that also contribute to decrease of the progression risk. Therefore, when assessing an individual patient, the natural evolution of BE towards EAC cannot be estimated only from cohort studies, but all the other aforementioned risk factors must be taken into consideration as well.

1.6. Barrett's Esophagus Management

There is a great deal of disagreements in contemporary literature regarding the management of BE. Above all, these issues reflect our inability to predict which patients will progress towards EAC, and to standardize our approach. Still, several management strategies are proposed, basically consisting of surveillance and

endoscopic treatment overlapping with chemoprevention and antireflux surgery.

1.6.1. Surveillance

Ever since the recognition of potential malignant transformation of BE towards EAC took place, screening and surveillance of potentially risky patients has been a mainstay strategy in management of Barrett esophagus. The rationale for this approach lies in the possibility to identify patients with GERD who have a higher chance of developing BE, to follow the BE patients' condition with a final goal to pinpoint the moment of dysplastic transformation or early neoplasia, and then to perform the necessary treatment.

The process of identifying those at risk among the GERD patients should include some of the following factors: duration of GERD symptoms, presence of hiatal hernia, age 50 or older, male sex, obesity, white race, tobacco use etc. Once identified, and histologically proven, BE patients are submitted to different surveillance protocols which differ in details among associations and their guidelines. [5, 38] Basically, for non-dysplastic BE, surveillance consists of regular endoscopic follow-up on every 3 to 5 years. It is recommended that endoscopic evaluation is performed with white light endoscopy. The specific biopsy protocol should also be utilized, consisting of four quadrant biopsies taken on every 2 cm of metaplastic epithelium, and separate biopsies should be taken from any mucosal abnormalities. Once established low-grade dysplasia, independent pathologist should confirm it. After verification of LGD, those patients should undergo endoscopic surveillance on every 6 to 12 months, again depending on different guidelines. The biopsy protocol should consist of taking four quadrant biopsies at every 1 cm of BE epithelium, and endoscopic mucosal resection of mucosal irregularities, such as lesions elevated from the level of surrounding mucosa. [38] At the time high-grade dysplasia is being recognized and confirmed, endoscopic surveillance should take place on every 3 months, unless some kind of eradication treatment is being employed. Different biologic markers are being introduced into studies and even clinical practice, but up to this moment none of the individual markers or panel of markers have shown efficacy in identifying those in higher risk for progression towards carcinoma. [42] So, none of those can be recommend in routine clinical practice, although the quest for identifying "the holy grail" among

the different biological markers which will be able to stratify patients in risk, remains the mainstay of different studies in the field of BE carcinogenesis.

If the end point of surveillance is to prevent EAC or to identify the early neoplastic changes, than the current surveillance protocols might be highly ineffective. There are several reasons for that. First of all, when identifying patients in risk for developing BE, clinicians starts from recognition of those having GERD symptoms. It is however well known that majority of patients with short-segment BE does not experience or does not report GERD symptoms. [43] Second important data is that approximately 40% of patients diagnosed with EAC have no prior history of GERD, and that less than 10% of those diagnosed with EAC was previously diagnosed with BE. [44] There are no clear literature evidences, that endoscopic screening and surveillance can reduce mortality from esophageal adenocarcinoma.

1.6.2. Medical Treatment of Barrett's Esophagus

Proton pump inhibitors (PPIs) are currently most efficient and most prescribed medications in the treatment of GERD. The PPIs are extremely potent in relieving the GERD symptoms as in the treatment of GERD complications e.g. erosive esophagitis. Their role in the maintenance therapy of patients with BE have also been proved. It has ben shown repeatedly that long term PPI therapy can slow the progression of BE metaplasia towards dysplasia and carcinoma. This specific effect of PPI medications can be achieved with substantial decrease in esophageal acid exposure, leading to reduction of inflammatory changes, such as promotion of mucosal healing and decrease of cell proliferation. [45] Therefore it seems reasonably to employ PPIs in the maintenance treatment of BE patients. A study conducted by Kastelein et al. showed that BE patients who underwent PPI therapy had decreased risk of neoplastic progression opposed to those taking histamine-2 receptor antagonists. [46] This was the Dutch cohort study, covering 540 patients, with a median follow-up of 5.2 years. It has been calculated that the risk of neoplastic progression for BE patients regularly taking PPI medications is decreased by 75%. Above all, it has showed that regular PPI therapy can even lead to regression of intestinal metaplasia, being observed on endoscopy as decrease of BE length and occurrence of squamous epithelium islets inside the BE epithelium. These studies however included small study population and the

evidences shown were strongly affected by inter-observer variation. A large meta-analysis was conducted with the intent to establish the potential role of PPIs in decreasing the risk of BE neoplastic progression.[47] The study included 2813 patients and 317 cases of EAC and BE associated HGD. The end-point of the study was that chronic intake of PPIs was associated with 71% decreased risk from neoplastic progression towards EAC and HGD. Opposed to this, a large case-control study by Hvid-Jansen and colleagues covered the population of 9883 patients with newly diagnosed BE. Median follow-up in this study was 10.2 years, during which 120 cases of EAC and HGD were confirmed. The relative risk for progression to HGD and EAC was higher in those patients who had lower compliance to PPI intake, than in those who took PPIs regularly.[48]

Although PPI treatment will improve symptoms and heal reflux esophagitis in majority of GERD patients, and also as mentioned before will decrease risk of neoplastic progression among BE patients, it will not affect the number of reflux episodes, but only change the acidity of refluxed content. This fact is extremely important in BE patients' treatment, considering the fact that bile salts have a special role in BE initiation and progression. It is somehow alarming that dramatic increase of EAC incidence, started when PPIs were introduced, in 1980s. Therefore, it has been hypnotized that PPI medications could change the natural course of GERD, shifting its spectrum of complications, from erosive disease and peptic stricture towards Barrett esophagus.[49] There is a rational explanation behind this. Chronic usage of PPIs elevates the average pH value in distal esophagus to >4 in a great majority of time over the 24h period. This is the pH value where bile salts may exhibit their potentially pathogenic role by affecting the cell junctions, and acting as signaling molecules. An experimental study on rats proved that gastric acid suppression with PPIs in the presence of duodenal reflux was associated with increased rates of intestinal metaplasia and molecular proliferative activity. A study employing sucrose permeability test in patients with BE showed that PPI intake was related with the higher degree of paracellular leak inside BE mucosa, whereas BE length did not make any statistical difference in the degree of leak.[50] This could implicate higher permeability of BE mucosa for the bile salts molecules in the pH>4 medium.

As a conclusion, although there are clinical evidence that could implicate beneficial role of PPIs in the treatment of BE, a novel studies evidences show a great deal of

concern for implementation of long term medical treatment for patients with BE.

1.6.3. Barrett's Esophagus and Antireflux Surgery

Among patients with GERD, those with BE are presented with higher incidence of LES dysfunction and ineffective esophageal motility, larger hiatal hernias, and more frequent and aggressive gastroesophageal reflux measured by 24 hour pH/ impedance testing. That is why those patients represent a special challenge to treat, both with intent to eliminate the GERD symptoms and stop the further alterations inside BE mucosa. Adequately preformed, antireflux surgery (ARS) can achieve both aforementioned goals. Utilization of ARS for patients with BE is getting a stronger role, when one knows that behind BE initiation and further progression is weakly acid and alkaline reflux, that cannot be stopped with medical therapy alone.

The positive impact of ARS on BE can be obtained in three modalities. The first is regression or lost of intestinal metaplasia, second is loss of dysplasia, or its regression to IM, and the third is induction of BE "quiescence", by which the possibility of further progression is being diminished.[51]

An often-cited study by Gurski et al followed 91 patients with BE IM and LGD out of which 77 patients underwent laparoscopic Nissen fundoplication (LNF). The criteria for regression were histologic confirmation of regression on two consecutive biopsies, 6 months apart, and all subsequent biopsies confirmation of IM or LGD loss. The histologic regression in ARS group was noted in 36.4% of patients, where 68% of LGD showed histologic signs of regression. The rate of regression was strongly dependent on BE length and time after surgery.[52]

A study by Cendes et al also highlighted the role of antireflux surgery in patients with short segment BE. In this study three different antireflux surgery types were evaluated, including duodenal switch, duodenal diversion combined with acid suppression and laparoscopic Nissen fundoplication (LNF). The study revealed high success rate in eradication of intestinal metaplasia, with overall success rate ranging from 60 – 65%. None of the surgical procedures employed showed superiority over the other two, so the authors concluded that LNF should be employed as the procedure of choice for patients with SSBE, as it was less invasive, and equally effective.[53]

The role of antireflux surgery in possible prevention of EAC was also evaluated. In the study, which evaluated 239 previously randomized patients, the late effects of medical therapy and ARS were assessed. [54] The study found no statistical differences between ARS and conservative treatment with PPI's in the term of late EAC incidence. The annual rate of progression towards EAC in this study population was 0.4%. The authors concluded that antireflux surgery could not be advised to patients with complicated GERD with the expectation of definitive relief from antisecretory drugs, nor could it be recommended as a protection against the EAC. A study by Lagergren et al also evaluated the effect of ARS on potential BE progression towards EAC. [55] The study population consisted of 218 cases of EAC and 820 controls. Among those diagnosed with EAC, 7 patients (3.7%) had undergone previous ARS. The study revealed that 4 of those 7 patients had used antireflux medications continuously. Hence, the authors postulated that occurrence of EAC after antireflux surgery may be due to the persistent reflux after ARS, meaning that fundoplication either disrupted in time or being inadequate at the first place. The systematic review of Chang et al dealt with the issue of potential protective role of antireflux surgery and medical therapy. The authors showed that the probability of progression in patients with antireflux surgery is 2.9%, and for those with medical therapy 6.8%. The authors found a statistically significant advantage of antireflux surgery over medical therapy with regard to the probability for BE regression.[56]

Along aforementioned studies, which have indicated that BE can regress after ARS in histological sense, there were also studies that highlighted the molecular changes in BE mucosa after antireflux surgery. Those studies showed reduced expression of p53 and COX1 after successful antireflux surgery. [57] The later finding may address to the quiescence inside BE epithelium, the one of proposed mechanisms of protective antireflux surgery role.

1.6.4. Endoscopic treatment

Despite several aforementioned modalities of BE surveillance, and reflux treatment with medications and ARS, the need for direct intervention and complete removal of BE epithelium persists among physicians. The complete eradication of BE mucosa is achievable, and effective with several endoscopic techniques.

Basically, all of those can be divided into two groups:

a) Endoscopic ablative techniques
b) Endoscopic mucosal resection

The aspects of all of these specific methods will be presented in the following text. However, in the quest for ideal endoscopic method for the eradication of BE, one must consider this procedure to be safe, effective and easily reproducible. It has been stated that ideal endoscopic procedure for BE eradication should have next attributes. Firstly, of all it needs to be feasible enough to be performed by any endoscopist skilled in interventional endoscopy. Secondly, the procedure must set the goal to eradicate BE completely. Thirdly, the endoscopic method must be safe, with minimal rate of procedure related complications, such as perforation, bleeding and stricture formation. At the end it must be well tolerated by patient, and of course if possible, it should be performed on the ambulatory one-day basis.[58]

There are several endoscopic methods used for BE mucosa eradication based on the principle of mucosal ablation. The ablation of the metaplasic or dysplastic epithelium is achieved by induction of the necrosis in the superficial mucosal layers. The optimal procedure should, however do minimal damage to deeper layers of esophageal wall, therefore minimizing the possibility of the potential complications. The usage of different ablative endoscopic techniques such as photodynamic therapy, laser ablation method, argon plasma coagulation, multipolar electrocoagulation, cryotherapy and radiofrequency ablation, has been presented in medical literature.

Photodynamic therapy (PDT) of BE is based on combination of systemic application of photosensitizer followed by endoscopic delivery of laser light energy over the whole surface of BE mucosa. The photosensitizer substance most often used is Porfimer sodium, which is usually being systemically injected 24 hour before the delivery of light energy. This substance is FDA approved in the USA for destruction of esophageal pre-neoplastic or early neoplastic lesions in the subset of patients who do not undergo esophagectomy. [59] In the study conducted by Faroulis it has been shown that PDT was highly effective in the treatment of HGD and intramucosal carcinoma with complete response rate around 80% at the 14 months follow-up. The most often occurred complications described in this study

where erosive esophagitis, photosensitivity and stricture of the esophagus requiring dilatation in 6% of patients. Overholt et al conducted randomized clinical trial for patients with HGD in which one group of patients was treated with PDT and omeprazole, while other group of patients received omeprazole treatment only. The early effects of study shown that PDT was more effective than omeprazole treatment only, in the term of lowering the risk towards EAC progression, whit 13% of patients receiving PDT and omeprazole together, and 28% of those submitted to omeprazole alone progressed to EAC, respectively. Study showed that PDT treatment was burdened with high rates of procedure related complications such as photosensitivity in 69% of patients and esophageal stricture in 36% of them. [60] Five years results of the same study revealed that 77% of patients who had underwent PDT therapy remained HGD free versus only 39% of those who had received omeprazole alone.[61] PDT has been proven method in the treatment of HGD, with satisfactory long term results, but obviously with high rates of complications, making this procedure not recommendable for broader population than those with HGD of intramucosal carcinoma, not suitable for esophagectomy.

Argon plasma coagulation (APC) is another ablative technique in use for complete eradication of metaplastic and dysplastic Barrett esophagus. It is a system, which delivers argon gas with trough the scope catheter. The gas is than exposed to monopolar electrode, resulting in dispersing heated gas stream, which is applied, on diseased mucosa. In the study showing late results of two randomized trials, the effects of APC treatment where better than surveillance only, in the term of reduced risk for BE progression. The studies were carried out on patients with non-dysplastic BE and LGD. One patient in APC group progressed towards HGD, versus three in surveillance group.[62] It has been reported that ideal candidates for APC treatment are patients with short segment BE and good control of gastresophageal reflux. This study reported relatively high incidence of subsquamous IM after APC treatment. [63] Factors contributing to recurrence of IM or subsquamous BE in this study were long segment BE and decreased dose of PPI medications. There is substantial amount of BE recurrence following the successful APC treatment of BE. This was noted in up to 66% of patients in a 15 months follow-up. [64]

There are no comprehensive literature reports showing utilization of laser

coagulation, cryotherpay and multipolar coagulation in ablative endoscopic treatment of Barrett esophagus.

The most applicable and worldwide exploited endoscopic ablation procedure is radiofrequency ablation with HALO system. This procedure will be presented and discussed in details in the following chapter.

Endoscopic mucosal resection (EMR) technique is a procedure used for staging and treatment of BE metaplasia and dysplasia. There are several different ways to carry out this procedure, including usage of submucosal injection followed with utilization of monofilament snare, cap or band ligation system. The basic advantage of the EMR technique over every ablation procedure is that it provides an adequate sample for accurate histologic staging. The biopsy sample obtained by EMR is usually 15- 20 mm in diameter, and sufficient enough to asses the resection margins. Therefore, it may be use either as therapeutic weapon, or a bridge towards more radical options, such as esophagectomy.

Its application is practically warranted in all cases of BE metaplasia or dysplasia presenting as nodal lesions. However, the limitation of the procedure is a surface area that can be treated, due to the high risk of postprocedural esophageal strictures. Even after successful mucosal resection of nodal lesions, the surrounding mucosa still carries the risk of neoplastic progression and therefore must be treated. That is the reason why currently we have numerous studies that finds combination of EMR and ablative techniques as a gold standard in the endoscopic treatment of BE with HGD.

A recently published study proved this standpoint. Smith et al evaluated 27 EMR specimens obtained from 21 patients underwent EMR for early EAC. The basic intent of the study was to evaluate presence of IM in those specimens. Although IM was not found in 10 out of 27 specimens, further analysis showed that in 3 of those it was present in previous EMR specimen, in 4 in previous biopsy specimens, and in 2 more in esophagectomy specimen following radical surgery. The authors concluded that IM is necessary predictor of EAC; therefore surrounding mucosa must be eradicated after successful EMR procedure.[65]

British authors presented their experience in combined HALO RFA and EMR endoscopic treatment of BE related HGD and intramucosal carcinoma (IMC). The study covered the 6-year period, ending in 2013, and included 500 patients from UK registry.[66] It has been shown that complete eradication or dysplasia and IM

rates improved over time, as evaluated in the second study period (2011- 2013). However, the risk of progression towards EAC remained 2- 4% over the whole study period, despite technical advances and higher success rate in eliminating the HGD.

Chadwick et al have made the comparison between effectiveness of EMR and RFA methods in the systematic literature review. The authors found similar eradication rates of dysplasia between two methods, although the majority studies included in the literature were single center experience, and only one made direct comparison between two techniques. Both methods proved to be highly efficient on a median follow-up of 23 months, with complete eradication of dysplasia being present on 95% and 94%, for EMR and RFA respectively. However, EMR studies revealed much higher rate of procedure related complications, such as esophageal stricture, which was present in 38% of EMR treated patients versus 4% of RFA patients.[67]

To conclude, EMR remains superior endoscopic treatment method for dysplastic nodular BE lesions in well-staged patients. The procedure must be accompanied with ablative technique, preferably RFA. This combined approach should guarantee superior treatment outcome, and minimize high rate of strictures resulting from EMR of broad field Barrett's mucosa.

1.7. HALO Radiofrequency Ablation of Barrett's Esophagus

You can hardly encounter more excitement about any new technology in the field of esophagology than the one following the introduction of the HALO radiofrequency ablation for the Barrett's esophagus. Surgeons and gastroenterologists dealing with this pathology gain strong, effective and safe weapon against the precancerous lesion, and made a lot of guidelines shifting in the BE management. With the number of HALO RFA procedures growing each day worldwide, issues emerge concerning its indications, durability and true effectiveness in the cancer prevention.

1.7.1. The Procedure Overview

HALO ablation system (Covidien, Sunnyvale, CA, USA) is based on radiofrequency energy which is delivered over the BE mucosa through the

catheters for circumferential (HALO 360) or focal ablation (HALO 90). [68] The principle behind this method is to deploy high power energy of approximately 300W, over the extremely short period of time (<300ms). The depth of the ablation is strictly defined, and if the procedure is followed there is a minimum risk of injuring the deeper esophageal layers. Approximately, depth of ablation is around 700μm, reaching to lamina muscularis of mucosa, but without extension towards submucosal. The electrode spacing is extremely narrow, less than 250μm, making the energy deployment even more superficial.

Besides regular flexible endoscope for upper GI endoscopy, the equipment for HALO RFA should contain the following: HALO energy generator, HALO 360 sizing balloons, HALO 360 balloons for energy deployment (various sizes ranging from 18 to 31 mm in diameter) or over the scope mounted catheter for focal ablation HALO 90.

Generally, HALO 360 system should be used for longer, circumferential segments of BE. HALO 90 is designed for focal ablation of shorter BE segments, and for treatment of residual fields of BE mucosa after treatment with HALO 360.

The process starts with inspection endoscopy and administration of 1% acetilcystein used for dissolution of mucus on esophageal wall. Getting the right landmarks for the measuring follows trough the course of procedure. Basic landmark is top of the gastric folds, and the chart for the procedure demand to write that landmark. The measuring of esophageal diameter starts 10 cm above that point. Before taking the endoscope out, the guidewire is introduced, and placed preferably through the pyloric channel. The HALO 360 sizing balloon is than open, calibrated and placed into the esophagus over the guidewire. The sizing is taking its course down the distal 10 cm of the esophagus, finish at the level previously set as the top of the gastric folds. After every measurement, one must write down the diameter of the esophageal lumen at that point, followed with the size of energy deployment catheter recommendation. The smallest recommended catheter should be utilized for the procedure. During one HALO 360 session maximal extent of treated mucosa should not exceed 6 cm.

After the measurement process, the ablation catheter is introduced over the guidewire, followed by endoscope. The balloon covers 3 cm, and after it is insufflated, and its position checked by endoscope, the energy deployment starts by pressing the pad connected to generator. The amount of energy deployed is

10J for metaplastic epithelium and 12J for dysplastic lesions. If the process of energy deployment was correct, it should be confirmed on the generator. The catheter is than placed 3 cm bellows the previous position, and the process is repeated. The overlapping of the two consecutive ablation fields should be minimal. The ablated mucosa is in form of white integument, which is being peeled down by endoscopic cap. After all of the mucosa was peeled down, the process of ablation is repeated over the whole area of treated mucosa. The characteristic appearance of well-ablated mucosa is in literature being described to be salmon colored.

The HALO 90 procedure starts also with the endoscopic inspection, followed by 1% acetilcystein washing of mucosal surface. The HALO 90 catheter is than mounted on the top of the endoscope in the manner that it resembles “baseball cap” on the monitor. Than the endoscope along with the catheter is being gently introduced into the esophagus, and care must be taken not to injure the pharyngeal area. The active electrode is than properly positioned, and afterwards deflected, therefore getting the optimal contact with the mucosal field, which is intended to treat. Two touches of the pad ensure that sufficient energy is deployed. Further, scraping of the treated mucosa is being performed with the electrode, after which the procedure is repeated, again until the salmon colored ablation filed appears.

The procedure is being carried out under conscious sedation (HALO 90), or in general endotracheal anesthesia for HALO 360 procedure. Duration of procedure is approximately 15- 20 minutes for HALO 90, and 20- 25 minutes for HALO 360. The procedure is well tolerated by patients and majority of them should leave the hospital same day, meaning that it can be routinely practiced on a ambulatory one-day basis.

1.7.2. Treatment Outcomes

One of the first studies performed during the development of HALO system was the one by Ganz et al. Authors applied HALO system on porcine model and on humans, planned for esophagectomy. The bottom line of this study was to precise the energy that needs to be delivered to achieve complete epithelial ablation, but without the injury of deeper esophageal layers. In this study energy levels from 8

to 12 J/cm^2 proved to be sufficient in achieving the mucosal ablation of the tissue that came into the contact with the electrode. These levels of energy density provide the ablation till the level of lamina muscularis mucosa. This experimental study showed that if the higher density energy was applied (20 J/cm^2), it has led to deeper submucosal injuries and subsequent stricture formation.[69]

Ablation of Intestinal Metaplasia (AIM) I multicenter trial was launched, for the treatment of patients with non-dysplastic BE. The trial was initiated with the intent to establish the proper doze of energy that need to be deployed. As a part of the AIM I trial Sharma et al. reported that 8 J/cm was insufficient for the majority of the patients and that satisfactory complete response rates were obtained with the energy levels 10 and 12 J/cm^2. [70] These results were followed by AIM-LGD trial were high success rates were showed in complete eradication of LGD and IM, 100% and 60% respectively, on a 1 year follow-up. The energy level was set on 12 J/cm^2. [71] On the two-year follow-up all patients had complete eradication of LGD and all but one had complete eradication of IM.

The studies, which followed, showed great results in complete eradication of BE dysplasia and IM, being over 90% in tertiary centers. For example, a study conducted by Fleischer et al presented that complete eradication of IM was achieved in 98% of patients on a 2.5 years follow-up.[72] However, the procedure gain wide popularity, due to its safety profile, and we have various literature reports coming even from community centers. In one such report by Lyday et al 429 patients were included from several community centers. The outcomes were highly satisfactory, without serious adverse effects, and low percentage of strictures (1.1%), where complete eradication of dysplasia was achieved in 89% of patients and IM in 72% on a median follow-up of 9 months. [73] This study could implicate high reproducibility of the procedure.

But the study that opened up the door for broad acceptance of the HALO RFA procedure was the one which results were published in 2009.[9] This was the multicenter randomized trial that included patients with non nodular, dysplastic BE, not longer than 8 cm. Patients were randomly assigned in 2:1 session to receive either HALO RFA treatment (up to 4 sessions) or an endoscopic sham procedure. The primary outcome variables were complete eradication of HGD, LGD and IM. The secondary study outcomes were to evaluate the rate of progression after RFA, and to establish whether the procedure can decrease the risk of neoplastic

progression. Overall, 127 patients underwent randomization, 84 receiving RFA treatment and 43 sham endoscopic procedure. Complete eradication of HGD was achieved in 81% of patients, and in 77.4% complete eradication of IM was achieved, significantly statistically higher than in sham control group. In the RFA group 3.6% of patients had disease progression in the course of the study, and the cancer incidence was 1.2%. The study showed that RFA treatment resulted high eradication rates of both dysplasia and IM, and reduced risk for disease progression.

Currently, most of the leading gastroenterological associations recommend HALO RFA as a treatment of choice for patients with histologically proven HGD, LGD, but not for those with IM, or for only selected IM metaplasia patients. The reason for this opinion is that procedure cannot guaranty lower risk for further progression in IM BE patients, and that it is not cost effective opposed to regular surveillance protocols. However, in the clinical practice, all three histological types are being treated with HALO RFA procedure, on daily basis. There are several why IM BE should be treated with HALO RFA, and the details for this will be presented in discussion part.

1.8. The Concept of Dilated Intercellular Spaces in GERD and Barrett Esophagus

In the context of current study a dilated intercellular spaces (DIS) will be evaluated in two separate manners. Firstly, to enlighten the role of DIS in the pathogenesis of BE, and secondly to present the literature data regarding the presence of DIS in neosquamous epithelium (NSE), with a special emphasis on the post RFA treatment.

The first time the dilated intercellular spaces were described in two independent papers was by Pope in 1978 and then by Hopwood in 1979. A paper published by Tobey et al. was the first to make a clear clinical connection between the DIS and gastroesophageal reflux disease, showing that the subject with erosive and non-erosive GERD had similar morphologic findings and the measurements of intercellular spaces, as opposed to healthy controls.[74] Therefore, DIS can be classified as microscopic feature of GERD. It has been postulated that DIS contribute to increased permeability of esophageal squamous cell epithelium

(SCE), and that heartburn will likely appear in the presence of DIS due to acid stimulation of the sensory nerves trough the frail epithelium. [49, 75] Once the H+ ions enter the highly permeable SCE, they led to sensory nerves stimulation and onset of heartburn or retrosternal pain. What also happens is that they induce the cell damage and in case of excessive long-term reflux, cell death. That is the mechanism behind the esophageal erosions formation.[76] It has been previously shown in rabbit experimental model that bile acids may contribute to DIS formation both in acidic or weakly acid environment.[77] In this study, bile acids in the acid solutions (pH 2), and weakly acid solutions (pH 5) lead to induction of increased permeability of esophageal mucosa and decreased transepithelial electrical resistance. Same study group conducted an investigation on 14 healthy volunteers, who were treated with infusion of acidic, weakly acidic, acid-bile and neutral infusions, administered 5 cm above the level of gastroesophageal junction. The biopsy specimens were taken after infusion treatment, and the presence of DIS was evaluated with electron microscopy. The study showed that both acid and weakly acid infusions provoked DIS without differences in the effect.[78] A recent publication by Ghatak et al explains the possible mechanism of bile salts acting as main pathologic determinant in DIS induction. [79] The study was based on in vitro formed cell cultures obtained from human tissue biopsies. These cell cultures were treated for 6 days with bile salts cocktails at pH 7.4, pH 5 and control media as well. The transepithelial electrical resistance was measured in the cell culture, as well as EPC1 cell growth. The morphologic status of three major junction complexes such as desmosomes, adherence junctions and tight junctions status was also observed by transmission electron microscopy. It has been shown that exposure to bile salts at pH 5 decreases the epithelial barrier function, causes the loss of stratification of SCE and lead to disruption of major epithelial junctions. The pH 5 media on which bile salts proved these pathogenic abilities resemble the one in patients with GERD treated with proton pump inhibitors. The study implicates that this acting of bile salts may play role in pathogenesis of refractory GERD. Previous study of Gathak et al. proved that bile salts have signaling effect on damaged esophageal epithelium, leading to the loss of differentiation and possible transformation towards columnar epithelium.[19]

So, what impact could bile salts and weakly acid reflux have on neosquamous epithelium (NSE), the one that develops after the successful RFA ablation? Jovov

et al published an important study regarding this issue in 2013. The study enrolled patients previously successfully treated with HALO RFA who underwent endoscopy with the biopsies of distal and proximal esophagus, as well as healthy controls.[80] Biopsy specimens taken from NSE revealed DIS and enhanced permeability trough the epithelium, in all of the 13 tested subject treated with RFA. Decreased transepithelial electrical resistance was also found indicating defective barrier function of NSE. Having in mind that all of those patients were treated with PPI on regular daily basis, and with knowledge of pathogenic role of bile salts in weakly acid media, which PPI's create, one must consider that behind morphogenic changes inside NSE is an ongoing weakly acid reflux.

The bottom line of our study is to show how the different post RFA treatment modalities will affect NSE, both in the term of IM and LGD regression as well as the recurrence, and in the term of morphological NSE characteristics, preferably by evaluating the presence of DIS after successful RFA treatment.

2. Aims

1. To evaluate the safety and efficacy of endoscopic radiofrequency ablation procedure in the term of early and late procedure related complications, as well as primary outcomes regarding the complete eradication of Barrett's esophagus.
2. To analyze the baseline data obtained from endoscopic and stationary manometry studies, with regard to their potential impact on the treatment outcomes.
3. To overview the potential factors contributing to the treatment feasibility and outcomes, especially having in mind potential recurrence of Barrett's esophagus after successful radiofrequency procedure.
4. To make the comparative analysis of different post RFA treatment strategies (proton pump inhibitors treatment on one hand, and laparoscopic antireflux surgery on other), with special emphasis on the their role on potential recurrence prevention.
5. To evaluate the efficacy of electron microscopy in obtaining the structure of neosquamous epithelium (the one that arose from ablated columnar Barrett's epithelium).
6. To analyze whether the post RFA treatment have the impact on neosquamous epithelium morphology.

3. Methodology

This was prospective clinical study initiated in November 2009 and conducted at the Department of Esophagogastric Surgery, First Surgical University Hospital, Clinical Center of Serbia, School of Medicine, University of Belgrade. The study was conducted in collaboration with the Institute for Histology, School of Medicine, University of Belgrade. Hospital Board and School of Medicine, Ethics Committee approved the study and the included patients gave their signed consent.

3.1. Inclusion and exclusion criteria

The following inclusion criteria were applied:

1. Histologicaly proven Barrett esophagus
2. Documented history of GERD> 5 years
3. Age 18 – 75
4. Family history of upper GI cancer
5. Obesity
6. Smoking

The first aforementioned criteria was obligatory, and patient had to met minimum 3 other criteria to be included in the study. If the case that entry histology was low-grade dysplasia (LGD), all of the other criteria were not taken into consideration and the patient advanced trough the study protocol. If LGD was present, second biopsy and pathohistological confirmation were obligatory.

The following exclusion criteria were applied:

1. Presence of reflux esophagitis LA gr C and D
2. Presence of peptic strictures of esophagus
3. Presence of caustic stricture or stricture of any origin

The prospective data base was created and it included: patients demographics, symptom score, data gained from upper GI endoscopy and esophageal stationary manometry, as well as the data related to HALO RFA procedure and post procedural follow-up. For the assessment of BE, endoscopy was performed in our Institution, with obligatory biopsies of columnar esophageal epithelium. Dedicated pathologist in the field of digestive pathology performed the patohistology

analyses. Prague C&M classification was used for the grading of BE extent. In this study we did not include patients with high-grade dysplasia.

3.2. Follow-up

Regular follow-up encompassed endoscopy 8 weeks after RFA procedure, and subsequent RFA in case of residual BE which was conducted three months after the first RFA session. Residual BE was diagnosed if there were macroscopic signs of columnar epithelium in tubular esophagus, confirmed with pathohistologic finding of intestinal metaplasia (IM) or LGD. Complete eradication of BE was considered if there were no signs of macroscopically present columnar epithelium in tubular esophagus, or evidence of IM or LGD on regular biopsy specimens which were taken separately from tubular esophagus and at the level of newly formed squamocolumnar junction (NSCJ).

We extracted those patients who had complete eradication (CE) of BE. These patients were further enrolled in follow-up protocol including endoscopies with biopsies on 6 months, 1 and 2 years. Patients having at least two control endoscopies with biopsies were classified in the group with complete follow-up data.

Further, those patients with CE of BE we divided in two groups according to the post RFA treatment. One group of patients was treated with daily PPI (Esomeprazol 40 mg per day) while others were submitted to laparoscopic Nissen fundoplication (NF) after or synchronous with RFA procedure. The allocation of patients was performed in following manner: Both treatments modalities after RFA (PPI therapy and Nissen fundoplication) were presented in detail to patients and were described to be equal both in the term of neosquamous epithelium protection and symptom control. So, basically, the post-RFA treatment modality was patient's preference.

Those patients having macroscopically visible columnar epithelium in tubular esophagus and histologicaly proven IM or LGD at any follow-up point were considered to have BE recurrence. If the macroscopically visible island of columnar epithelium was noted at the level of tubular esophagus it was consider as recurrence, regardless of histology obtained. If the macroscopically visible columnar epithelium was found at the level of NSCJ or as a columnar tongue

above the NSCJ, the pathological confirmation of IM or LGD was obligatory parameter for confirmation of the recurrence. For those patients data were taken regarding the recurrence timing, as well as pattern of recurrence on tubular esophagus or NSCJ. If present in follow-up biopsies, subsquamous intestinal metaplasia was separately noted.

3.3. HALO RFA procedure

The RFA procedure was conducted with HALO 90 and 360 systems (HALO 360 or HALO 90, Covidien, Sunnyvale, CA, USA). HALO RFA system consists of generator unit, which produces energy in dose of 10-12J/cm2 (10 J/cm2 was used for patients with IM, and 12 for patients with LGD) and specially designed catheters. The energy was applied trough the catheters covering approximately one fourth of esophageal circumference (HALO 90), or whole esophageal circumference (HALO 360). Procedure was carried in analgosedation for HALO 90, and general endotracheal anesthesia for HALO 360 procedure. Maximal extent of circumferentially treated BE mucosa in one RFA session was 6 cm. If residual BE was detected, next RFA session was performed with minimum 3 months apart from the last procedure. The details of the procedure are presented in the introduction section.

3.4. Antireflux surgery

Laparoscopic Nissen fundoplication was performed in a standardized fashion by the same surgical team. Patient was placed in dorsal position, legs spread apart. Operating surgeon is positioned between patient's legs. First 10 mm port was placed with Hasson open approach, 2 cm left of the midline, at the middle of distance from umbilicus to processus xifoideus. Three working ports are placed , followed with Nathanson liver retractor. Briefly, pars densa of hepatogastric ligament was dissected, and right crus of the esophageal diaphragmatic hiatal orifice was visualized. Esophagus was dissected, encircled, and after a hiatal closure and dissection of short gastric vessels "floppy" Nissen fundoplication was created with three non-absorbable sutures and without the utilization of the bougie. In case that anatomy of gastric fundus was favorable, Rosseti modification of

Nissen fundoplication was performed, without dissection of short gastric vessels. Synchronous RFA with HALO 360 was performed after complete dissection and encirclement of the esophagus, and HALO catheter was positioned under the combined vision of laparoscope and endoscope. Usually, in those cases type II esophageal dissection was necessary in order to get the sufficient abdominal length of esophagus.

3.4. Transmission electron microscopy analysis of intercellular spaces in epithelium of eosophageal mucosa

A separate analysis of biopsy specimens was obtained from patients in whom complete eradication of BE was achieved with one, or several RFA sessions. The basic inclusion criteria were CE-IM and minimal period from the last RFA session of 6 months.

Endoscopic biopsy specimens were taken with “jumbo” forceps. In each patient two specimens were obtained from neosquamous epithelium (3 cm above the distal margin of palisade vessel zone, or 3 cm above the top of the gastric folds) and from proximal esophageal segment (5 cm below the level of upper esophageal sphincter).

Biopsy samples were fixed with 3% glutaraldehyde in cacodylate buffer and postfixed in 1% OsO4. After dehydration in graded alcohols, cells were embedded in Epoxy medium (Sigma-Aldrich, 45345). Thin sections were mounted on copper grids (Sigma-Aldrich, G4901), and stained with uranyl acetate and lead citrate for examination on an electron microscope (Morgagni

268D, FEI, Hillsboro, OR). The sections and micrographs for the analysis were selected by using Systematic Uniform Random Sampling. [81] The intercellular spaces were measured as distances in between 80 cells, on 10 micrographs per sample at 2800x magnification by the use of TEM. The same micrographs were used for fractional volume analysis as previously described using the following formula: volume fraction = $\sum PAP / \sum Pcyt \times \rho$, where $\sum PAP$ is the number of points of a dense grid counted on intercellular spaces, $\sum Pcyt$ the number of points of a second grid counted within the cytoplasm, and ρ is the number of points on the dense grid that represent each point of the grid used for the cytoplasm (in this case 25).

3.5. Statistical analysis

Data are expressed in mean and median values. Fisher's exact test, Student's t-test and Kruskal-Wallis one-way analysis of variance test were utilized. We used one-way ANOVA as well. Logistic regression was used to assess to assess the potential predictors of BE recurrence and the potential predictive factors for the development of the dilated intercellular spaces. Point of statistical significance was set on 0.05. For the purpose of statistical analyzes SPSS, version 20.0.0. was used.

4. Results

4.1. Demographics

There were overall 56 patients who met eligibility criteria and entered the study. Out of this number majority were males, 39 patients (69.6%). There were 17 female patients included (30.4%).

Mean age of the patients at the moment of their inclusion in the study was 47.3 ± 10.8, ranging from 18 till 74. Majority of patients were older than 50 years, 39 of them (69.6%)

There were 42 patients who had previous history of smoking (75%), out of those 29 (51.8%) were active smokers at the moment of inclusion.

Mean value of body mass index (BMI) of included patients was 29.7 ± 6.1. 32 patients (55.3%) had their BMI in range from 25 – 30. BMI over 30 was present in 11 patients (19.6%).

Family history of upper GI cancer was reported by 4 patients (7.1%). Esophageal adenocarcinoma was not reported in family history by any patient. All four of the patients reported family history of gastric cancer.

The duration of GERD symptoms was observed in the 3, 5 and 10 year period. Duration of GERD symptoms > 3 years was present in 48 (85.7%) of patients. In 41 of them GERD symptoms were present for more than 5 years, and in 23 (41.1%) GERD symptoms were present for more than 10 years.

History of antisecretory drugs utilization was reported by 53 patients (94.6%). Majority of those had prior history of proton pump inhibitors intake, which was present in 46 patients (82.1%).

The basic demographic data and the data related to GERD history are presented in table 1.

Table 1. Basic demographic and clinical data

№ of patients	56
Male to female ratio	39 / 17
Mean age	47.3 ± 10.8
BMI	29.7 ± 6.1
Smoking history	42 (75%)
Anamnesis of family upper GI cancer	4 (7.1%)
Anamnesis of > 3 years of GERD symptoms	48 (85.7%)

4.2. Preprocedural upper GI endoscopy, esophageal stationary manometry and Barrett's histology

Upper GI endoscopy was performed in our Department before the procedure in all of the patients included in the study. We utilized the Prague C&M classification. According to those mean value of M length in this patients group was 4.3 ± 2.1, ranging from 1 to 12. Mean value of C length was 3.1 ± 1.4, ranging from 0 to 10. (Table 2.)

Hiatal hernia was present in 49 patients (87.5%). Hiatal hernia of 3 and more cm in size was present in 25 (44.6%) of the patients. In this subgroup, 19 patients had type I hiatal hernia, while in 6 of them type III hiatal hernia was present.

Erosive esophagitis was present in 19 (33.9%) of patients at the study entry. According to LA classification grade A esophagitis was present in 12 (21.4%), and LA grade B in 7 of patients (12.5%). Healing of esophagitis prior to HALO RFA procedure was obligatory endoscopic criteria.

The entry BE histology was obtained in all patients. Intestinal metaplasia (IM was present in 38 (67.9%) of patients, while 18 patients had proven low-grade dysplasia (LGD). (Table 2., Figure 1.) Regarding the entry histology distribution of the patients, there were no statistical differences in the term of age, gender or BMI.

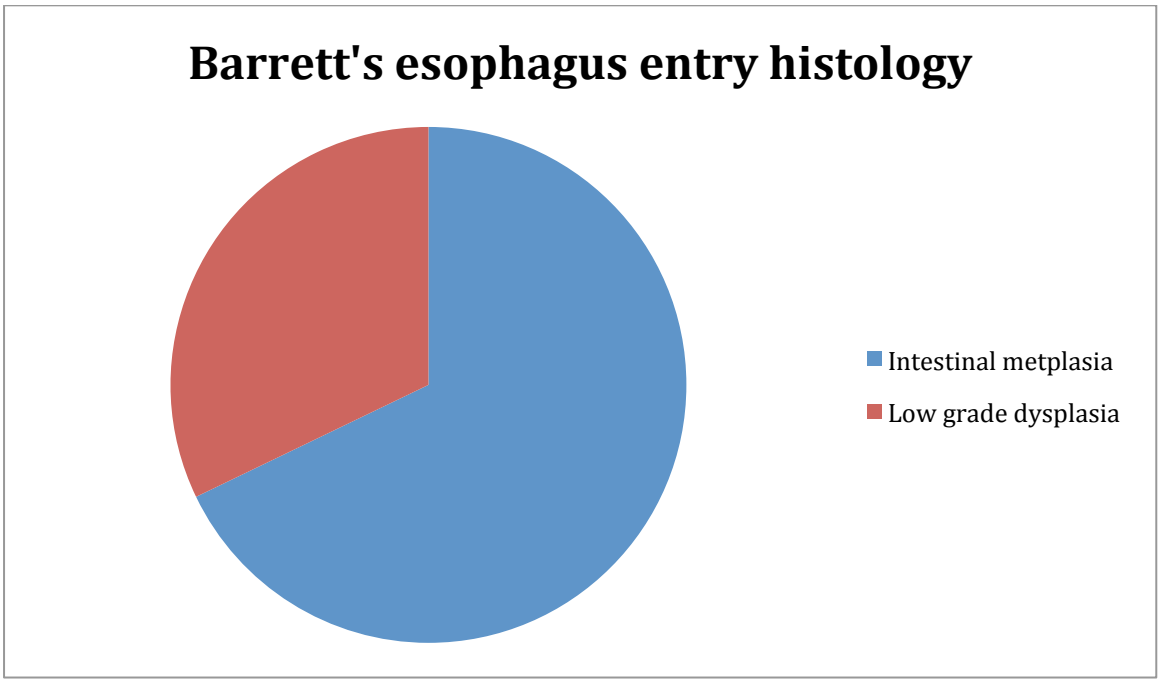

Figure 1. Stratification of Barrett's esophagus patients according to the entry histology

In patients with IM, hiatal hernia>3 cm was present in 14 patients (36.8%), and in 11 patients who had LGD (61.1%). There was a statistically significant higher incidence of larger hiatal hernias in patients with LGD opposed to those with IM ($p=0.021$, $p<0.05$). Mean value of M length in patients with IM was 4.4 ± 2.0. Mean value of M length of BE in LGD group was 4.1 ± 2.3. There was no statistical difference in the term of M length of BE between patients with IM and LGD. Mean value of C length in patients with IM was 3.3 ± 1.4. Mean value of C length of BE in LGD group was 2.8 ± 1.2. There was no statistical difference in the term of C length of BE between patients with IM and LGD.

Mean value of lower esophageal sphincter pressure measured by stationary esophageal manometry was 4.2 ± 2.9 mmHg. Ineffective esophageal motility (IEM) was present in 37 (66.1%) of the patients. With regard to IEM classification, 29 patients had amplitudes of distal esophageal contractions <30mmHg, 5 were presented with non -propulsive contractions, and 3 with low amplitude simultaneous contractions as major motility disorder (Figure 2).

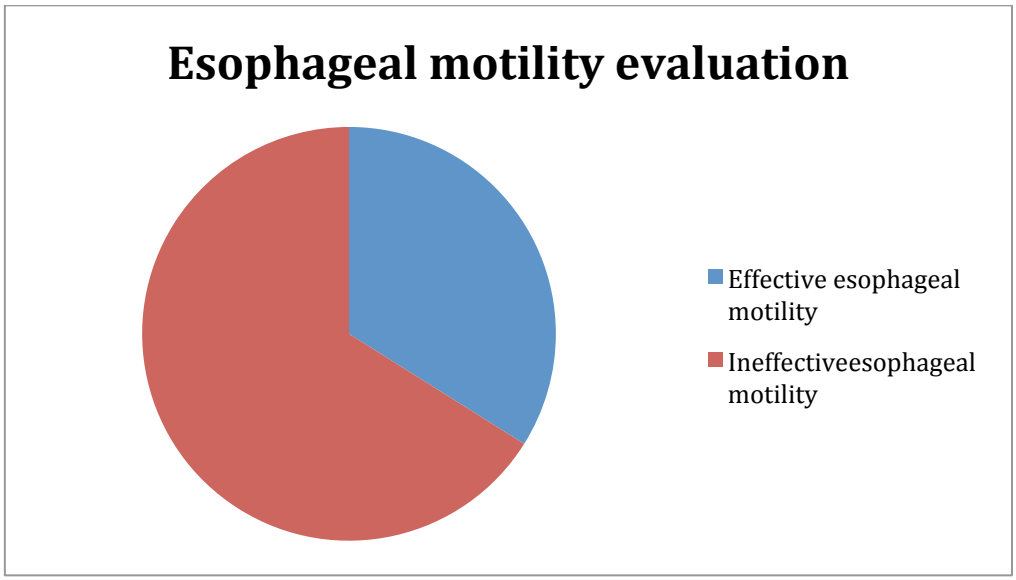

Figure 2. Stratification of Barrett's esophagus patients according to the effectiveness of the esophageal motility measured by stationary esophageal manometry

Regarding the BE histology, mean value of LES basal pressure among patients with IM was 4.3 ± 3.1 mmHg and mean value of LES basal pressure among patients with LGD was 4.1 ± 2.6 mmHg. IEM was present in 22 patients (57.8%) with IM, and in 15 (83.3%) with LGD.

There was no statistical significance regarding the mean values of LES basal pressure between patients with IM and LGD. There was a statistically significant higher incidence of ineffective esophageal motility in patients with LGD opposed to those with IM ($p=0.017$, $p<0.05$).

Table 2. Preprocedural upper GI endoscopy, esophageal stationary manometry and Barrett's histology data

No of patients	56
median M length (cm)	4.3 ± 2.1
median C length (cm)	3.1 ± 1.4
BE histology IM	38 (67.9%)
BE histology LGD	18 (32.1%)
presence of hiatal hernia	49 (87.5%)
Pts with HH > 3 cm	25 (44.6%)
mean lower esophageal sphincter pressure	4.2 ± 2.9 mmHg
Pts with ineffective esophageal motility	37 (66.1%)

4.3. Data related to HALO RFA procedure

HALO 360 RFA procedure was applied as a primary procedure in 20 patients, while in 36 primary procedure choice was HALO 90.

Mean duration of the HALO 360 procedure was 24.1 ± 3.4 minutes. Mean duration of HALO 90 procedure was 18.3 ± 3.4 minutes. All of the HALO 360 procedures were performed in general endotracheal anesthesia. HALO 90 procedure was performed in analgosedation in 25 patients and in general endotracheal anesthesia in 2 patients.

There were no procedure related complications, both in HALO 360 and HALO 90 groups of patients.

Most common side effects of the HALO procedure were: post procedural chest pain, which was present in 39 patients (69.9%). Mean duration of postprocedural chest pain was 2.3 ± 1.2 days. Post procedural dysphagia occurred in 21 patients (37.5%). Mean duration of dysphagia was 11,5 ± 6.1 days, and it resolved spontaneously in all patients. Mean length of hospital stay after RFA HALO procedure was 1.2 ± 0.2 days.

The majority of the patients (30 patients, 53.6%) received additional HALO procedure for residual segments or islands of BE. HALO 360 RFA was employed in 11 patients as a second choice procedure, and in two of them as a third choice procedure. HALO 90 RFA was employed as a second line treatment in 23 patients, in 10 patients as a third line treatment, and in 2 patients as a fourth line treatment. One patient received 5 HALO RFA sessions.

In one patient where HALO 360 for LGD C1M3 was applied in 12J/cm2 regimen stricture developed, but without need for dilatation. This patient was treated with proton pump inhibitors after the procedure.

The data regarding the number and type of HALO RFA procedure, and side effects of HALO RFA procedures are presented in tables 3. and 4.

Table 3. HALO RFA – type and number of procedure

Choice of procedure	HALO 360	HALO 90
Primary HALO RFA procedure	20	36
Second HALO RFA procedure	11	23
Third HALO RFA procedure	2	10
Fourth HALO RFA procedure	0	2
Fifth HALO RFA procedure	0	1

Table 4. HALO RFA related side effects

HALO procedure side effects	Chest pain N	Dysphagia N
Incidence N (%)	39 (69.9%)	21 (37.5%)
Duration (days)	2.3 ± 1.2	11,5 ± 6.1

4.4. Laparoscopic Nissen procedure

Laproscopic Nissen fundoplication (LNF) was performed in 22 patients.

There was no intraoperative complications, or conversions to open procedure.

The mean duration of LNS was 56.1 ± 11.4 minutes.

There were no postoperative intrahospital complications. Mean duration of hospital stay 2.8 ± 1.1 days.

In 4 patients LNF concomitant with HALO 360 procedure was performed. There were no procedure related complications. The mean duration of concomitant LNF/RFA procedure was 85.4 ± 24.8 minutes.

The most common adverse effect of LNS was postoperative dysphagia which was reported by 18 patients (81.8%). 10 (45.4%) of those had dysphagia that lasted up to 10 days, in 6 patients (27.2%) duration of dysphagia was up to one month, and in two patients (9.1%) it lasted up to three months.

There was no need for medical interventions due to severe or persistent LNF related dysphagia.

Second most common adverse effect of LNS was postoperative bloating which was present in 13 patients (59.1%). In 7 patients (31,8%) bloating symptoms resolved up to six months after surgery. In 6 patients (27.3%) bloating symptoms persists up to study conclusion. Those patients are under follow-up, and receive prokinetic medications.

4.5. Endoscopic follow-up

Out of the primary included 56 patients, complete resolution of BE was obtained in 47 of them (83.9%). Regarding the BE histology complete eradication of IM was achieved in 33 patients (86.2%), and in 14 patients with LGD entry histology (77.7%).

Analysis of 9 patients in whom complete BE eradications was not achieved was made.

There were 5 patients with IM (55.6%), and 4 patients with LGD (44.4%) in whom complete response was not obtained. Second HALO RFA session was performed in 8 of them. In one patient, only single HALO 360 procedure was performed due to pronounced post procedural esophagitis and poor compliance to medications prescribed.

In 5 patients residual BE was presented in form of multiple islands of BE mucosa, in 3 as a residual circumferential BE segment, and in 2 combination of residual circumferential segment and multiple islands of BE.

Extreme dilatation of the esophagus was seen in one of these nine patients (11.1%). This patient had morbid obesity as well, with BMI being measured at the study entry 46.1. After two sessions of RFA patient was put on list for gastric by pass surgery, which was successfully performed afterwards.

Other baseline patient's characteristic such as age, gender, GERD duration, hiatal hernia size, presence of IEM did not significantly statistically differ from the baseline characteristics of the whole study population. (Table 5.)

Table 5. Baseline characteristic of patients with residual BE

№ of patients	9
Male to female ratio	6 / 3
Mean age	42.9 ± 15.1
BMI	33.8 ± 9.6
Smoking history	7 (77.8%)
Anamnesis of family upper GI cancer	0
Pts with HH > 3 cm	5 (55.6%)
Pts with ineffective esophageal motility	4 (44.4%)

4.6. Stratification according to post RFA treatment

Patients with complete eradication of BE were divided according to the study protocol in two groups regarding the post RFA treatment option.

There were 25 patients treated with PPI after HALO RFA, and 22 submitted to laparoscopic Nissen fundoplication (LNF). Out of 22 patients who underwent LNF, 4 were operated concomitant to first HALO RFA session.

In the group of patients receiving PPI's treatment after HALO RFA, there were 17 males and 8 females. Mean age of this study group was 48.1 ± 12.4. Mean value of BMI in this group was 30.1 ± 3.4. Mean number of HALO RFA procedures in this group was 2.3. IM was present in 18 and LGD in 7 patients. Median values of C and M length were 2.9 ± 1.1 and 3.9 ± 1.7, respectively. Hiatal hernia bigger that 3 cm was present 12 (48%) of the patients. (table 6, figure 3)

Table 6. Demographic, endoscopic and RFA related data in patients with CE BE distributed in two groups regarding the post RFA treatment

	HALO RFA + PPI's	HALO RFA + LNF*	p value
No of patients	25	22	
mean age	48.1 ± 12.4	45.4 ± 15.2	0.677
gender (male to female ratio)	17/8	15/7	0.768
BMI	30.1 ± 3.4	28 ± 4.1	0.341
mean No of RFA procedures pp*	2.3	2.1	0.451
BE histology (IM/LGD)	18/7	15/7	0.243
BE C length	2.9 ± 1.1	2.6 ± 1.3	0.407
BE M length	3.9 ± 1.7	3.8 ± 1.4	0.377
HH* size > 3 cm N(%)	12 (48%)	12 (54.4%)	0.385

In the group of patients who underwent LNF treatment after HALO RFA, there were 15 males and 7 females. Mean age of this study group was 45.4 ± 15.2. Mean value of BMI in this group was 28 ± 4.1. Mean number of HALO RFA procedures in this group was 2.1. IM was present in 15 and LGD in 7 patients.

Median values of C and M length were 2.6 ± 1.3 and 3.8 ± 1.4, respectively. Hiatal hernia bigger that 3 cm was present 12 (54.4%) of the patients. (table 6, figure 3)
There were no statistical differences in term of gender distribution, age and mean BMI between the groups (table 6, figure 3).
Also, there were no statistical differences regarding the mean number of RFA procedures employed, BE histology (IM/LGD ratio), C and M length of BE, as well as percentage of patients with hiatal hernia size > 3cm. (table 6, figure 3)

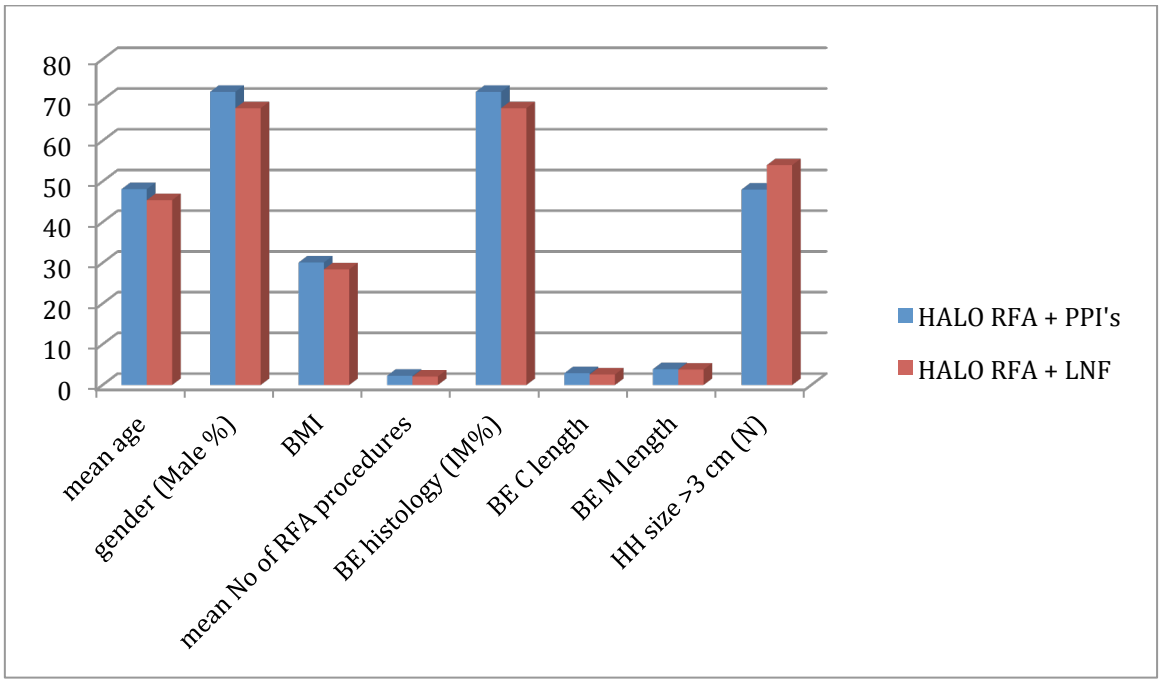

Figure 3. Comparison of demographic, endoscopic and RFA related data in patients with CE BE distributed in two groups with regard to the post RFA treatment

4.7. Recurrence of BE

The criteria for complete follow-up were met by 40 (85.1%) of those with complete eradication of BE. The remaining 7 patients had one follow-up endoscopy with biopsies after CE-BE. After 2 year of prospective follow-up BE recurrence was noted in 5 patients (20%) treated with PPI's, and 2 patients (9.1%) who underwent LNF (figure 4). In those treated with PPI's we marked 4 recurrences of IM and one LGD. Both patients who had recurrent BE in LNF group had IM.

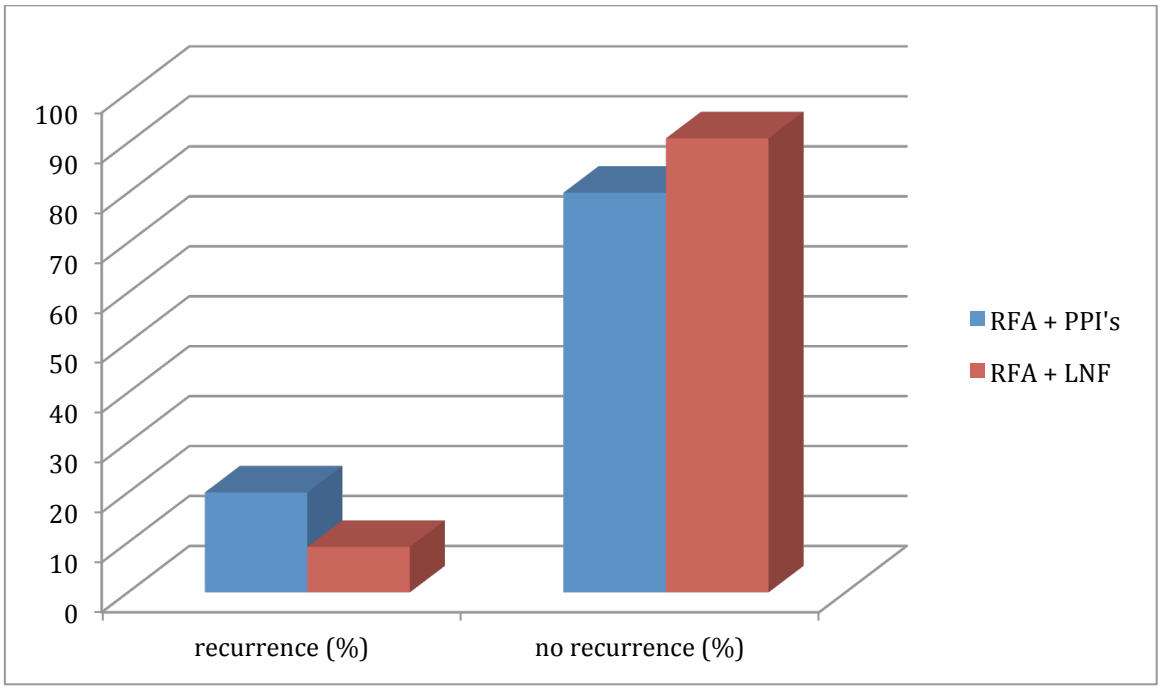

Figure 4. Distribution of the patients with regard to the type of post RFA treatment and incidence of BE recurrence

With regard to the recurrence timing, in PPI's group recurrence was marked in 1 patient after 6 months, 2 after one year and in 2 after two years. Mean time from last RFA session till recurrence detection in PPI's group was 482 days (range from 173 to 812 days). In LNF group 2 patients were found to have recurrent BE on 2 years surveillance endoscopy, in one it occurred after 730, in other 851 days after last HALO RFA session. (Table 7.)

Table 7. Timing of recurrence according to different post RFA treatment modalities

	RFA + PPI's	RFA + LNF
6 months	1	0
1 year	2	0
2 years	2	2

With regard to pattern of recurrence, in PPI's group in 4 patients recurrent BE was noted both in tubular esophagus and at the level of NSCJ. Also it was present in two or more biopsy specimens in all 5 patients with recurrent BE.

In LNF group one recurrent BE was noted at the level of tubular esophagus, and one on the level of NSCJ. In one patient it has been proven in more than two biopsy specimens. (Table 8.)

We found no recurrence of BE underneath neosqouamous epithelium (this was also confirm in selected subgroup of patients after evaluating jumbo forceps biopsy samples on electron microscopy, see Results chapter: Morphological characteristics of neosquamous epithelium).

Table 8. Pattern of recurrence according to different post RFA treatment modalities

	RFA + PPI's	RFA + LNF
Tubular esophagus	1	1
NSCJ	0	1
Tubular esophagus+NSCJ	4	0

Comparison was made between the groups regarding the overall incidence of the BE recurrence. There was no statistical difference between the incidence of BE recurrence regarding the post procedural treatment option (p=0.423, p>0.05). (Table 9.)

Table 9. The recurrence of BE after RFA in patients treated with PPI's or Nissen fundoplication in 2 year follow-up

	no recurrence	recurrence	p value
HALO RFA + PPI's n(%)	20 (80%)	5 (20%)	0.423
HALO RFA + LNF n(%)	20 (90.9%)	2 (9.1%)	

Different demographic patient's characteristic and patients endoscopic baseline characteristics were also evaluated with regard to recurrence of BE. Gender was not shown to be statistically significant in the term of recurrence (p=0.768, P> 0.05), as well as age (p= 0.677, p>0.05). This was also case with hiatal hernia size (cut-off value>3 cm), BE C&M length, p values: 0.385, 0.407 and 0.377, respectively. (Table 10.)

Table 10. Demographic and baseline endoscopic characteristics influence on overall rate of BE recurrence after HALO RFA and with regard to post procedural treatment

	RFA + PPI's	RFA + LNF	p value
Gender			0.768
Male (%)	68.0%	63.6%	
Female (%)	32.0%	36.4%	
Mean age (years	47.4	46.0	0.677
HH> 3 cm (%)	44.0	59.1	0.385
BE C length (mean, cm)	2.9	2.6	0.407
BE M length (mean, cm)	3.9	3.8	0.377
Recurrence, n(%)	5 (20%)	2 (9.1%)	0.423

When we extracted the patients with BE C&M>4 cm we found statistical difference between the groups in favor of LNF group ($p= 0.021$, $p<0.05$). All the patients with BE recurrence in PPI's group had C length of BE >4 cm. In LNF group 8 patients had C length of BE > 4 cm, 2 of those had recurrent BE after 2 years. (Table 10)

Table 11. The recurrence of BE after RFA in patients with BE C length≧4cm treated with PPI's or LNF

	no recurrence	recurrence	p value
HALO RFA + PPI's n(%)	0	5 (100%)	0.021
HALO RFA + LNF n(%)	6 (75%)	2 (25%)	

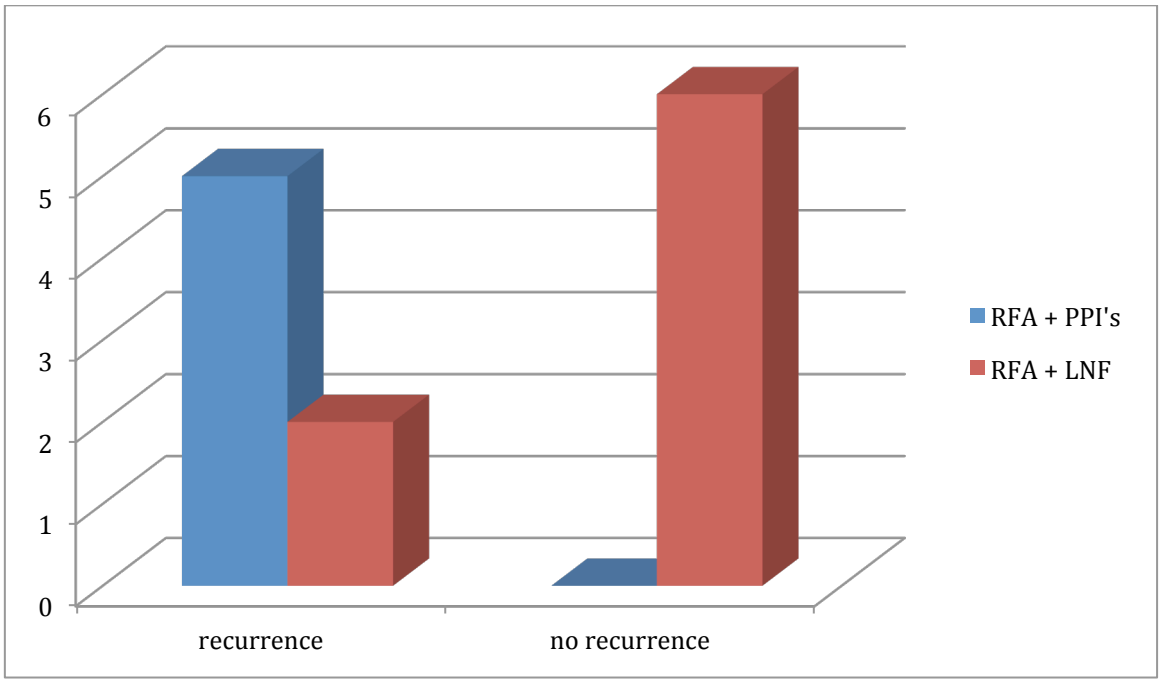

Figure 5. *The comparison of the recurrence rates of BE after RFA in patients with BE C length 4cm with regard to the post RFA treatment*

We extracted the factors which had a strong correlation with the BE recurrence after HALO RFA. Those were size of hiatal hernia (cutoff >3 cm), and the length of BE (C&M criteria). (table 11)

Table 12. Factors influencing recurrence of BE after RFA

	no recurrence	recurrence	p value
Hiatal hernia size			0.012
<3cm (%)	85.7%	14.3%	
>3cm (%)	70.8%	29.2%	
BE M length (mean, cm)	2.4	4.4	<0.001
BE C length (mean, cm)	3.2	5.6	<0.001

4.8.Transmission electron microscopy

Overall, 20 patients in which CE-IM was achieved underwent specific biopsy protocol for transmission electron microscopy evaluation. In 11 of them complete biopsy procession for TEM was made, and the data were obtained.

The basic demographic data, the data regarding the upper GI endoscopy and stationary manometry at the study entry are shown in the table 13. There were no differences in the term of age, gander, nor in the term of the mean length of BE segment or mean values of lower esophageal sphincter.

The mean value of IS length in the patients with CE-IM under the PPI's regimen was 0.734μm ± 0.325 and 1.262 ± 0.174 μm in the proximal esophagus and NSE, respectively.

The mean value of IS length in the patients with CE-IM who underwent antireflux surgery was 0.378 ± 0.116 μm and 0.879± 0.329 μm in the proximal esophagus and NSE, respectively.

With the regard of the proposed cut-off value for the non pathologic length of IS, all of the patients treated with PPI's had DIS in NSE, and 4 out of 5 had DIS at the level of proximal esophagus. In the group of patients who underwent antireflux surgery 3 patients had DIS in NSE (50%), and 2 of them had DIS in proximal esophagus (33.3%).

The comparative analysis of the IS lengths was made according to the post RFA therapeutic regimen. We found no statistical difference among the mean IS values in NSE, although there was statistical tendency favoring the antireflux surgery.

The mean values of IS length in proximal esophagus were statistically significantly lower in the group of patients who underwent antireflux surgery.

The mean values of IS length inside NSE and proximal esophagus are presented in table 13.

Table 13: Mean values of IS length with regard to type of treatment and biopsy site

	Prox. Esophagus (mean, μm)	Dist. Esophagus (mean, μm)
RFA + LNF	0.378 ± 0.116	0.879± 0.329
RFA + PPI's	0.734 ± 0.325	1.262 ± 0.174
P value	.003	.06

Fractional volume analysis showed that percentage of IS with the regard to whole thickness of NSE in the group of patients treated with PPI's was 27.82%. (range from 20.1 till 38.9%). The percentage of IS inside the squamous epithelium of proximal esophagus was 16.93% (range from 11.58% till 20.48%).

Fractional volume analysis showed that percentage of IS with the regard to whole thickness of NSE in the group of patients undergoing antireflux surgery was 22.23% (range from 15.52 till 42.57%). The percentage of IS inside the squamous epithelium of proximal esophagus was 15.35% (range from 12.86% till 18.03%).
No statistical differences were found when IS volume percentages were compared between the groups of patients with different post RFA treatment.

5. Discussion

Barrett esophagus remains filed of great controversies, starting from its definition, over the course of disease evolution, till the issue of treatment. Still, somehow it seems that with the introduction of HALO system things change towards better, as we gained effective weapon to safely treat BE. Although showed to be extremely effective, HALO is still relatively new method, and years must pass, before medical public generates definitive judgment.

This study addressed the patients with intestinal metaplasia and low-grade dysplasia. The intent of the study was to show how effective and durable is HALO RFA in complete eradication of IM and LGD. The second basic study goal was to evaluate the effect of post RFA treatment regarding the disease recurrence prevention, with special emphasis over morphologic characteristic of neosquamous epithelium.

The inclusion criteria of the study were created with the respect of so far proven risk factors for BE, such as GERD, age>50, obesity and smoking. Basically, all of the patients included in the study were those with long lasting GERD, with the median GERD symptom duration over 7 years. The percentage of those with significant hiatal hernia was also high, over 60%. The obesity was present in over 20%, while majority of patients had their BMI between 25- 30. There were no differences in the term of gender in our study population.

Endoscopic data revealed high percentage of those with long segment BE, with median C values being over 3 cm, and median M values over 4 cm. Median value of lower esophageal sphincter basal pressure was approximately 4 mmHg in this study, and the majority of patients had marked ineffective esophageal motility.

These data are in concordance with the majority of studies showing baseline characteristics of BE patients. It has repeatedly being shown that BE patients as a subset of GERD cohort, have lowest values of LES, poor esophageal motility, and more pronounced acid and weakly acid reflux measured by pH and pH impedance monitoring. Among the other risk factors hiatal hernia size was shown to be not only the risk factor for developing the long segment BE, but also an independent risk factor for EAC. [82] A large meta-analysis that included 4390 patients with BE found that patients with hiatal hernia had increased risk for long segment BE, even after adjusting risks for reflux and BMI. [83] A study by Savarino et al evaluated patterns and intensity of reflux in patients with erosive esophagitis, short and long segment BE, as well in healthy controls. The authors employed 24 hour

pH/impedance monitoring and found that among aforementioned groups patients with long segment BE had highest scores on 24 pH monitoring and most defective acid clearance opposed to other groups. The subgroup patients with long segment BE had significantly more episodes of both acid and non-acid acid reflux, seen on impedance tracings than other observed groups.[84] Also, it has been documented that the BE is the end stage chronic GERD disease, and that vast majority of BE patients suffer from some kind of esophageal motility impairment, most frequently observed as low amplitude contractions of distal esophagus, and non propulsive peristaltic waves.[85]

When it comes to assessment of esophageal motility in BE patients, one must ask a question whether this is a primary disorder leading to more pronounced GERD, or it is a consequence of chronic exposure of esophagus to pathologic reflux. This issue was evaluated in two studies from our Department. We have initially showed that patients with ineffective esophageal motility (IEM) can be operated safely with total Nissen fundoplication, without concern of persistent dysphagia. [86] In the same study it has been shown that esophageal motility can recover after antireflux surgery, which may be partially induced by complete reflux blockage, and healing of esophagus. However, in the second publication a special emphasis was given upon subtypes of IEM.[87] It was shown that some of those patients do not have benefit from antireflux surgery, in the term of motility recovery, and the conclusion was that the origin of IEM might be different among three subtypes. So, high incidence of IEM among BE patietns could have two implications. Firstly, the long lasting GERD causes metaplasia of espophageal epithelium, and leads to more deeper injuries of esophageal wall, leading to ineffective esophageal motility, which on the other hand contributes to severity and frequency of GER. Secondly, if IEM exists combined with BE, than the treatment with PPI's will probably be insufficient for these patients, as it will not improve esophageal clearance, nor will stop the weakly acid reflux.

So far published data showed high efficiency and safety of HALO radiofrequency ablation in the treatment of BE. The results of our study matched these findings. During the study period, there were no complications related to the procedure, such as perforation or bleeding. The most common adverse effects were retrosternal chest pain and dysphagia, which completely resolved in 10 days after the procedure. The rate of the stricture was also extremely low. The stricture was

present in only one patient, and it did not require endoscopic dilatation, or other kind of invasive intervention. The patient complained of mild dysphagia, which was treated satisfactory with PPI's in combination with prokinetics. Another stricture was noted in one young patient with 12 cm long BE segment, which had developed esophageal stricture prior to the RFA procedure. The stricture was at the level of proximal thoracic esophagus and it did not progress after the initial HALO treatment. The area of stricture was not treated with HALO 360 initially, due to inability for adequate balloon calibration. After LNF and initial HALO RFA procedure were performed, second HALO RFA lead to complete resolution of BE.

The so far published studies with long-term follow-up of patients after HALO RFA also point out a high safety of the procedure. In the study, which evaluated the durability of HALO RFA for patients with dysplastic, BE, authors also presented the data regarding the safety profile of procedure.[88] These data have even more value, because the study covered the patients with dysplasia, which is more demanding group to treat. In this study, authors noted 4 serious adverse effects (3.4%). Those were: one serious upper GI bleeding in patient undergoing antiplatelet therapy, and three overnight hospitalization due to the onset of chest pain. The perforation rate was zero. However, authors reported that 7.6% of patients developed esophageal stricture, which was diagnosed with endoscopy, with or without dysphagia. In all patients dysphagia resolved, with mean 2.8 dilatation sessions per patient. In the study of Velanovich post RFA strictures occurred in patients with extremely long segment BE, more than 12 cm in length in 3 cases, and more than 6 cm in one case. [89] The author calls for special attention in those patients. According to Fleischer among the published series utilizing the HALO treatment, chest pain seems to be most common side effect, and it usually lasts for several days. In 1- 2% of patients chest pain is more intense and eventually will require readmission hospitalization. Esophageal strictures occur rarely, and usually are related with previous esophageal mucosal resection. In the review of over 20 000 cases, incidence of esophageal strictures was 1%, and no mortality related to HALO RFA procedure was recorded.[72]

Controversies still persists regarding the application of HALO RFA procedure for patients with non-dysplastic BE. There are observations that there are no evidences to justify this, because the risk of progression in patients with IM is low, and surveillance strategies should be employed. This standpoint is more

supported with the data indicating substantial risk of BE recurrence and presence of buried glands after RFA, visualized with novel endoscopic visualization techniques. [90, 91] There is even a study showing that RFA for non-dysplastic is not cost-effective in term of reducing the risk of progression. Same study proved the cost effectiveness of RFA in the treatment of LGD and HGD. [92] The rationale for RFA in non-dysplastic BE can be supported with the following facts, well analyzed in the review paper of Akiyama et al. [93] First, it is impossible to foresee which patients will progress to HGD and EAC, and the timing of progression trough the course of disease is unpredictable. Second, there is a risk of misdiagnosis due to the sampling error, interobserver disagreement and poor compliance with surveillance protocols. Third, the physicians must be aware of the anxiety and cancerophobia in patients diagnosed with BE. It is sensible to propose RFA procedure to patients with non-dysplastic BE, after the careful evaluation of aforementioned facts, and the risks present in each individual patient. The safety profile, efficacy and proven decrease of risk progression, favor the RFA procedure over surveillance protocols, and justify it's implementation in patients with non-dysplastic BE.

One of the basic goals of our study was to evaluate the efficacy of HALO RFA procedure in complete eradication (CE) of intestinal metaplasia and LGD. During the course of the study CE of BE was found in 86.2% and 77.7%, for IM and LGD, respectively. We tried to analyze the possible causes of residual BE. In 4 patients the explanation behind failed RFA could be dilated distal esophagus. These patients were offered with concomitant HALO/LNF, but they have chose PPI's treatment instead. It is the opinion of our group that patients with dilated esophagus cannot be successfully treated with RFA, because the RFA electrode will not have proper contact with the mucosa. In one patient with long segment BE, HALO 360 procedure did not lead to satisfactory outcomes, although this patients did have normal diameter of the esophageal lumen. The poor compliance with the medications could be the potential cause in this case. In others, ongoing reflux despite therapy, or in one case even LNF, was probable cause of failed RFA.

When evaluating the extent of BE, especially in its most distal segments and in the presence of hiatal hernia, one must have in mind the concept of "dilated distal esophagus" (DDE), proposed by Chandrasoma and DeMeester. [94] According to this logic and well-proved standpoint, the proximal extent of gastric folds and

cardia are in fact dilated esophagus, transformed during time into the columnar lined transition zone. In the presence of GERD, this “dilated distal esophagus” is often being misinterpreted as proximal stomach and gastric folds, although it probably is columnar lined esophagus. The only method by which one can righteously make a proper judgment is to take the biopsy samples from this region. Cardiac mucosa and oxyntocardiac mucosa should be “proclaimed” as the dilated end- stage esophagus. Trough this concept one can explain the evolution of the progressive esophageal dilatation in patients with the long segment BE. Those patients represent technical challenge for RFA. The “DDE” idea was nicely evaluated in review paper published by Lenglinger and coauthors. [95] They went beyond historical misinterpretation of the so-called “cardia” region, and nicely structured the article regarding the true origin of esophagogastric junction mucosa. If the peritoneal coverage is absent and submucosal glands are present, it is obvious we are dealing with esophagus, covered with columnar epithelium. Thus, the concept of DDE is so far the best elucidated and documented explanation of BE origin. With the introduction of RFA, this concept could be explanation beyond the ineffective ablation and high incidence of recurrent IM at the level of cardia.

Of course, regardless of esophageal dilatation, the most acceptable explanation for residual BE after RFA is the ongoing and uncontrolled reflux. The study by Krishnan et al. evaluated this aspect in details. [96] This study included 37 patients with long segment BE IM, and patients with HGD. After the 3 consecutive ablation sessions, patients were divided in the groups with complete response (CR) and incomplete response (ICR). 24-hour pH/impedance data showed that those with ICR had significantly higher number of weakly acid and weakly alkaline reflux opposed to those with CR. Since all patients were given PPI’s in a twice-daily regimen, the number of acid reflux episodes did not differ between the groups. Same study recognized the size of hiatal hernia and length of the BE to be independent predictive factors for incomplete response after RFA. Study by Akyama et al was in concordance with these data.[97] It was shown that patients with normal or mild intraesophageal acid exposure have significantly higher probability of achieving CR after RFA opposed to those with moderate and severe esophageal acid exposure, measured with 24-hour pH metry. Along with the acid exposure, size of hiatal hernia was recognized also as an independent risk factor for RFA failure. Some other studies have identified size of hiatal hernia and length

of the BE as the most important factors for successful RFA procedure.[98] If we return to concept of “dilated distal esophagus”, and we have in mind literature proofs of negative effect of hiatal hernia on RFA outcome, the rationale for antireflux surgery seems to be inevitable. This attitude is even more justified with the aforementioned evidences of ongoing weakly acid and weakly alkaline reflux despite PPI’s therapy.

To conclude the discussion about the possible causes of incomplete response after RFA for BE, we have to emphasize three basic factors. Those are: ongoing reflux, DDE together with hiatal hernia size and length of BE.

The role of antireflux surgery as an adjunct to RFA procedure, or as a choice of post RFA treatment has not been properly addressed in the literature so far. Up to now, there are no randomized trials comparing the role of antireflux surgery and PPI’s on RFA outcomes. There are several publications until now, however, which are valuable to mention and which cover this issue.

One of those is for sure study conducted by O’Conell and Velanovich. [99] The authors included 47 patients in whom RFA was performed for non-dysplastic and dysplastic BE. Of those, 19 patients had their fundoplication created before, after or concomitant with RFA. The remaining 28 patients were treated with PPI’s. After one-year follow-up, the persistent BE was present at one patient with fundoplication, and at 7 in whom PPI’s treatment was employed. The patients who had fundoplication required lower number of RFA sessions to achieve complete eradication of BE, and the majority of them require only a single session. In a small study population, Eldaif et al reported 100% complete response rate after RFA for IM and LGD. In this report, 11 patients were treated afterwards with fundoplication, but due to persistent reflux symptoms during PPI’s treatment, and no direct comparison on the RFA outcomes were made [100] Recent report by Johnson and colleagues failed to prove advantage of post RFA LNF over PPI therapy in terms of BE recurrence prevention. In this study complete eradication of IM remained at 70% of patients after fundoplication. However, authors did postulate that fundoplication after RFA could be a superior option in preventing the further disease progression. [101]

A superior role for antireflux surgery in the light of RFA procedure could be in patients with large hiatal hernias, and dilated distal esophagus. This could be obtained by employing concomitant laparoscopic antireflux procedure and RFA.

Goers et al first described this approach in the literature.[102] Their study was conducted in 8 patients, of which 6 was presented with major hiatal hernia requiring reduction. The procedure was concomitant HALO 360 RFA during the laparoscopic fundoplication. RFA was performed after hernia reduction, and esophageal encirclement, which affected the esophageal lumen in the manner that RFA electrode applied more closely to the mucosa. This kind of approach showed that in 5 patients complete eradication of BE was achieved after a single RFA session, while the remaining three patients underwent consequent ablation session. The rates of procedure related complications were substantially high. One patient in this small study group developed stricture, and in one esophageal perforation occurred. The cause of perforation may be related to the fact, that in this particular patient BE segment was too long to be treated in a single session.

In our study, concomitant LNF and RFA procedure was proven to be safe. We have treated 4 patients in this manner during the course of the study. Procedure was not too time consuming, and it was performed in conjunction with the basic rules of HALO 360 procedure. Most importantly the esophageal calibration was performed after the hiatal hernia had been reduced, esophagus encircled and pulled down. That way the potential pitfall of choosing wrong diameter of RFA balloon was avoided. Second, no more than 6 cm of BE mucosa was treated in the single session. There was no procedure related complications in our study.

Another interesting observation, which we couldn't document so far, is that patient with concomitant or prior fundoplication, have faster mucosal recovery after RFA, observed on the first endoscopic evaluation. It has been noticed in our study that patients in whom PPI's are employed in a twice-daily regimen after RFA, have very fragile mucosa 8 weeks after procedure, when first endoscopy is usually scheduled. This mucosa is sometimes covered with superficial erosions. In this subset of patients we usually add evening dose of H2 blocker, along with previous PPI therapy. The higher number of patients is needed to document these observations, and more standardization in the term of interobserver agreement.

We have shown the data regarding the efficacy of RFA treatment, and rates of BE complete eradication. Now we must focus on reports regarding the recurrence of BE after successful RFA treatment. The observation must be made upon three relevant issues:

1. rates of recurrence
2. timing of recurrence
3. pattern of recurrence

The issue of recurrence rates was well assessed in the meta-analysis conducted by Orman et al. [103] This study included the literature data obtained from prospective publications, published before 2011 and in which method of ablation was HALO RFA. The studies employing additional ablative treatment or mucosal resection were not taken into consideration. Further, all of the included studies needed to have data concerning the rates of complete response in the term of IM eradication (CR-IM) or dysplasia eradication (CR-D). Minimum 12 months follow-up was necessary inclusion criteria. The studies, which were subject of this meta-analysis, came from USA and Europe. There was overall 15 full text articles and 5 abstracts after the exclusion criteria were applied. Those were the studies reporting efficacy, durability and combination of those two. The efficacy analysis showed that CR-IM was found in 78% of patients (ranging from 70 – 86%), and CR-D in 91% of patients (87 - 95%). During the treatment 9 patients progressed to EAC, with the estimated risk ratio of 0.1%. Overall recurrence rate of IM after initial CR-IM was 13% (9 - 18%), and it was higher in low quality studies. Recurrence of dysplasia was noted in 5 patients, and EAC in 4 patients when analyzing the included studies.

Maybe more comprehensive recurrence analyzes was published by Gupta et al. [91] This study included patients treated in three referral tertiary centers in the USA. There were overall 592 patients, of which 71% had HGD as baseline histology. This is the largest prospective study so far, including patients with dysplasia in whom RFA treatment was undertaken. In the course of the treatment during 22 months from first RFA session CR-IM was noted in 56% of patients. Out of those patients with CR-IM during the two-year follow-up recurrence of BE was noted in 33% of patients. In the majority of patients recurrence was histologically noted as IM, while in 22% recurrence of dysplastic epithelium occurred. The recurrent cases were managed endoscopicaly, but no data were provided regarding the future outcome of these patients. In our study, recurrence rate among patients with CR-IM after two-year follow-up was 14.9%. This value could be implicated with the sampling error, especially in the term of cardia region biopsy. However, the

patients with visible islands of columnar epithelium on the level of tubular esophagus were proclaimed to have recurrence, even without histologic confirmation.

An issue that is hard to address at this point is the timing of recurrence after achieving the CR-IM. This is due to the fact that this procedure is generally new, and longer follow-up period is needed. The studies addressing timing of recurrence after RFA so far, reported in majority of cases 1 and 2 -year follow-ups. For example, Shaheen et al report on 3 years follow-up of their group previously included in the AIM randomized control trial. [104] The CR-IM was achieved in 75% and CR-D in more than 85%, without maintenance RFA. The validity of these data is confirmed with the confirmation that depth of biopsy specimens was adequate (subepithelium present) in 82.4% of patients. [105]

We tried to analyze the study follow-up period trough the 6 months, one year and two years follow-up. The recurrence was marked in two patients inside first six months of follow-up period, two inside one-year and three more inside two-year follow-up period. Interestingly, earlier occurrence of recurrence was noted in patients who were treated with PPI's, opposed to those who underwent antireflux surgery. The statistical analysis of this finding was not achievable due to the limited number of patients, and imprecise methodology process, nevertheless it is worth wile mentioning. Further analyzes may be forwarded to precise timing of recurrence detection with the evaluation of factors contributing to recurrence, especially the impact of post RFA treatment.

Pattern of recurrence is another important issue that needs to be evaluated. It was stated by Korst et al that recurrence of BE after RFA treatment my occur in three separate modalities, as the recurrence at the level of:

1. tubular esophagus
2. gastroesophageal junction
3. beneath neosquamous epithelium (subsquamous recurrence, or "buried glands)[106]

In this study authors followed overall 151 patient treated with RFA for metaplastic/ dysplastic BE for a median period of 18 months. The group of patients with persistent/recurrent IM after RFA treatment was recognized. There were overall 26% of patients with persistent/recurrent IM. Most commonly in this study group recurrence was identified at the level of gastroesophageal junction (10 patients).

Both subsquamous presence of IM and recurrent IM at the level of tubular esophagus were recognized in three patients. In the retrospective report from Vaccaro et al, 47 patients in whom CR-IM was achieved underwent further surveillance. [107] Patients were followed for median 13.3 months (5- 38 months), with median 2 surveillance endoscopies (range 2- 5) performed at that period. The study reported overall rate of recurrent IM to be 31,9%, with cumulative yearly incidence being 25.9%. Pattern of IM recurrence in this study was as follows: 73% of patients had recurrent IM at the level of gastroesophageal junction, while 27% of patients had recurrent IM at the newly recognized islands of columnar epithelium on the level of tubular esophagus. The authors draw the conclusion that close surveillance after CR-IM with RFA is essential due to very high cumulative one-year rate of recurrence. One must be careful when evaluating this study for two reasons. Firstly, the high frequency of recurrence detection at the level of gastroesophageal junction may be due to the sampling error at the first place, meaning that CR-IM was not achieved at all. Secondly, when performing HALO RFA one must take special caution at the GEJ level, because HALO RFA technique gives us ability of "creator", the one that forms the new squamocolumnar junction. So, with the proper evaluation of the GEJ, the primary ablation procedure should probably be extended towards top of the gastric folds, and should cover the whole region of so called cardia. This is the point when we should think again about the "dilated distal esophagus" concept, and think trough the method of ablation again.

Gupta et al. also evaluated the pattern of recurrence in the previously cited report. [91] Out of the 37 patients with recurrent IM and dysplasia, in this study, it was almost equally found at the level of tubular esophagus and GEJ. The subsquamous recurrent IM was not reported in this study. In the dysplasia AIM sham control trial, incidence of "buried glands" in those with CR-D was approximately 5%. [9]

The incidence of subsquamous IM after RFA is rarely being reported. Grey et al conducted a large systematic review of the literature with the intent to evaluate the frequency and significance of subsquamous IM in patients previously treated with ablative endoscopic procedure. [108] The review covered PDT and RFA methods. After 22 reports employing PDT were evaluated, the incidence of "buried glands" found to be 14.2%. RFA treatment was assessed trough 18 literature reports, in

which 9 patients with proven “buried glands” were found, overall 0.9%. Dutch authors conducted an interesting study in order to properly establish the incidence of buried glands after RFA treatment. [109] They used the term “pseudoburried glands”, for those obtained in biopsy samples from macroscopically visible islands of columnar epithelium. The 69 consecutively RFA treated BE patients were included in this study. The biopsy samples were taken from normal appearing squamous epithelium, and from visible islands of columnar epithelium on tubular esophagus. The incidence of subsquamous IM in normal appearing squamous epithelium biopsy samples was 0.1%. Biopsy samples of columnar mucosa islands revealed high incidence of “buried glands”, which were found in 21% of patients. Authors conclude that sampling error must be avoided when reporting the true incidence of “buried glands” after RFA.

The application of the novel endoscopic visualization methods may be helpful in identifying the areas of NSE with buried gland hidden underneath. One of such methods is an optical coherence tomography (OCT). The basis of this new technology is to provide visualization of the 3D tissue ultrastructure. In the study applying OCT technology in patients with CR-IM after RFA, the incidence of buried glands was stunningly 63%. [90] The fact that majority (approximately 70%) of buried glands were found at the level of SCJ, may rise an issue over the adequacy of ablation in that area.

In our study the recurrence was equally found at the level of GEJ and tubular esophagus. We did not found recurrence beneath normally appearing NSE. The biopsy specimens were taken with jumbo forces biopsy, where lamina propria of mucosa could be evaluated in majority of biopsy specimens. These findings were confirmed when electron microscopy was performed in selected group of patients with CR-IM.

The other part of this study was based on the electron microscopy evaluation of the biopsy specimens taken from neosquamous epithelium. The included patients had complete eradication of previous BE IM or LGD achieved in one or more sessions of RFA. The idea for such investigation came from study conducted by Jovov et al. [74] This study implicated that NSE is a fragile epithelium with dilated intercellular spaces and increased permeability. The low expression of claudin-4, valuable brick inside tight junctions was also confirmed in NSE. These findings were observed in all included patients, previously treated with RFA and in which

CR-IM was obtained. The paper by Orlando addresses this publication in details. [110] Author brings his concern of the increased permeability and defective barrier functions of NSE. If NSE would be exposed to pathological acid or even weakly acid reflux, and with regard to its weakened defense mechanisms, the recurrence of BE may occur. Also, if the buried glands are present beneath NSE, increased permeability trough the epithelium could initiate their genetic instability. What emerges, as a relevant issue is whether these NSE morphologic characteristics are in its true nature, or possible provoked by ongoing reflux.

The basic intent of our study was to asses the impact of ongoing reflux on NSE. The patients included were treated with PPI's and antireflux surgery after CR-IM achieved with RFA. The rationale for this methodology was under the premise that fundoplication will mechanically block gastroesophageal reflux, regardless of its acidity. Therefore, we might establish whether there are morphological changes inside NSE, and to sought out the potential role of ongoing reflux.

The neosquamous epithelium is a product of re-epithelization after successfully performed ablative endoscopic procedure. The basic histology of NSE resembles the one of native squamous epithelium. One to two basal cell layers, followed by multiple layers of more mature cells, form NSE. In the early post-ablation phase, hyperplasia of the basal layer and lamina propria papilla elongation may be present, indicating the regenerative reflux induced changes. [111] The origin of NSE is not clearly established. It is noted that it may originate from new post ablation SCJ, or from the remnant cell laying underneath of BE, and/or from esophageal glands. [112] Paulsen et al proposed that NSE and BE have their origin in the same progenitor cell. [113] Most importantly, the rigorous evaluation of the NSE in patients that had neoplastic and preneoplastic lesions, showed no genetic abnormalities or buried glands, which were proven in the BE epithelium prior to RFA. [114]

However, the finding of the DIS in all NSE biopsy specimens in patients with CR-IM in the study by Jovov et al warrants further investigation regarding the defensive properties of NSE. [74]

Dilated intercellular spaces are generally accepted as the microscopic characteristic of GERD. The studies on patients with erosive and non-erosive esophagitis, and healthy controls, proved that DIS is present in GERD regardless of macroscopic findings. An interesting study by Calabrese et al proved that DIS is initiated not only by acid reflux but with bile reflux as well. In their study there were

no differences regarding the width of intercellular spaces in GERD patients in whom predominant acid or bile reflux was recorded (2.27 ± 0.47 μm in patients with pure acid reflux and 2.11 ± 0.23 μm in those with mixed bile/acid reflux). Authors indicated that ultrastructural damage of esophageal epithelium may occur even with adequate acid suppression therapy, due to the noxious effect of bile salts. [115]

The aforementioned study of Jovov et al showed that biopsy samples obtained from the level of proximal esophagus had not revealed DIS in the majority of patients (11/13). This finding implicated that DIS was present almost exclusively in NSE, but not in the untreated proximal esophagus. These findings are not in concordance wit the data obtained in the study by Caviglia et al. [116] The authors conducted the measurement of intercellular spaces in NERD patients and healthy controls in biopsy samples taken from the distal and mid thoracic esophagus. Interestingly, in NERD patients, DIS were found equally in distal and proximal esophagus, indicating that pharyngeal presentation of reflux in NERD patients may be elucidated by this finding. The bottom line of this study may be, that if pathologic reflux is present, it will not make ultrastructural damage only at the level of distal esophageal segments, but proximal as well.

The results of our study implicated presence of dilated intercellular spaces in NSE. Although there are no standardized data, which could serve as the basis for comparison, for each patient proximal healthy esophagus, was also analyzed, and the control values were obtained. Mean value of intercellular spaces (IS) length obtained from NSE in all patients under PPI regimen was 1.262 ± 0.174 μm, while mean length of IS in proximal esophagus in the same group of patients 0.734 ± 0.325 μm. Mean value of IS length in biopsy specimens of patients who underwent LNF was 0.378 ± 0.116 μm and 0.879± 0.329 μm in proximal esophagus and NSE, respectively. When comparative analysis was made for each individual patient, comparing the values of IS length in NSE and proximal esophagus, statistical difference was reached in 5 out of 11 patients. These results implicates that the presence of DIS was found in the majority of patients after RFA inside the NSE. However, with the lack of standardization, one cannot make an assumption how these values should be interpreted, especially when compared to samples of proximal esophagus in individual patient. We are usually referring to the IS length in the proximal healthy esophagus to be normal. If the values obtained from NSE

segment do not differ from proximal, than it is hard to pronounce the presence of DIS in that individual patient. But, we also have to be aware that reflux may have high extent, which may lead to DIS in proximal esophageal segments. It is our opinion that analysis of DIS must be made with reference to proposed cut-off value, and certainly not with presumption that length of IS in proximal esophagus is normal.

Comparative analysis of IS lengths in NSE were made between those who underwent antireflux surgery, and those receiving PPI's therapy. The mean IS length was statistically significantly smaller in the NSE of the patients who underwent antireflux surgery, opposed to those who were treated with PPI's. In accordance to applied cut off value of IS length, all of the 5 patients in the PPI's group were found to have DIS in NSE. Out of 6 analyzed patients in group of patients who underwent surgery, 50% had DIS in NSE. Further, significantly higher values of mean length of IS values were found in biopsy specimens obtained from proximal esophagus of patients inside PPI's group. This finding may implicate that ongoing reflux in these patients does not lead only to DIS in distal, but proximal esophagus as well. These data are matched with those presented in the study of Caviglia et al in which DIS were confirmed in proximal esophagus of NERD patients. [116]

The limitations of the current study are obvious. The first limitation is of course limited number of patients, which is directly influenced by the very demanding study methodology. This limitation was at least partially overcome with high number of data obtained from each biopsy sample. The internal checking of data for every biopsy sample was performed with presentation of three-dimensional model, which provided the even more valuable data in the term of overall surface that IS occupy regarding the whole thickness of epithelium. The second limitation is that patients were not stratified according to the baseline characteristics, prior to RFA treatment, which could make an impact on the results.

The conclusion may be as follows. Dilated intercellular spaces are present in NSE, but not to that extent earlier described. Further, there is a statistical evidence that DIS are present in less extent in those who underwent antireflux surgery after RFA, of course more patients and longer follow-up is warranted. Presence of DIS in the proximal esophagus of the majority of post RFA patients undergoing

continuous PPI's treatment, even more justifies the hypothesis that ongoing weakly acid reflux is the probable etiologic factor behind epithelial damage.

When these data are combined with those obtained from endoscopic follow-up and basic histology, we might get better insight into RFA perspective in the term of future treatments protocols.

It seems to be a necessity to standardize the criteria regarding the post RFA treatment, and to provide the straightforward indications for antireflux surgery after ablation. Based on our study and the existing literature data, we could make a following proposition.

The patients who should be proposed with antireflux surgery after HALO RFA are those with long segment BE, hiatal hernia> 3cm, and those with documented ongoing acid and weakly acid reflux.

6. Conclusions

1. Radiofrequency ablation (RFA) is safe and effective procedure in complete eradication of Barrett's esophagus.

2. Patients with Barrett's esophagus in this group were commonly presented with long lasting GERD, hiatal hernia and ineffective esophageal motility, as well as insufficient values of lower esophageal sphincter.

3. Patients with dilated distal esophagus, hiatal hernia and those with uncontrolled gastroesophageal reflux represent the group in whom complete eradication of Barrett's esophagus is difficult to achieve with RFA, with high percentage of residual BE.

4. After the evaluation of the complete sample of patients none of the post RFA applied treatment types did not reach the statistical advantage in the term of BE recurrence prevention. Nissen fundoplication had significantly better protective effect over PPI's in the group of patients with long segment BE and hiatal hernia >3 cm.

5. Electron microscopy is highly efficient in evaluation of morphological structure of neosquamous epithelium.

6. Dilated intercellular spaces are present inside neosquamous epithelium, as well as in proximal macroscopically healthy esophagus. DIS are present in higher extent in those patients undergoing medicamentous treatment opposed to those treated with antireflux surgery, both in distal and proximal esophagus.

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List of Abbreviations

BE	Barrett's esophagus
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
EAC	Esophageal adenocarcinoma
GI	Gastrointestinal
BMI	Body mass index
LNF	Laparoscopic Nissen fundoplication
LES	Lower esophageal sphincter
GEJ	Gastroesophageal junction
SCE	Squamous cell epithelium
NSCJ	Newly formed squamocolumnar junction
RFA	Radiofrequency ablation
NSE	Neosquamous epithelium
DIS	Dilated intercellular spaces
IM	Intestinal metaplasia
LGD	Low grade dysplasia
HGD	High grade dysplasia
CE- IM	Complete eradication intestinal metaplasia
CE-D	Complete eradication dysplasia

Biography

Ognjan M. Skrobić, MD, MSc was born in Banja Luka, Bosnia and Hercegovina in 1978. There he finished elementary and high school. He graduated at the School of Medicine, University of Belgrade in 2003. After going through the residency training in general surgery the Clinical Center of Serbia, School of Medicine, University of Belgrade from 2005 till 2010, he received his Surgery Board Certification on 2010. In 2010 he received his Master of Science title, after the public defense of the thesis entitled „Significance of the symptomatology in the treatment and evaluation of the patients with the gastroesophageal reflux disease“. In 2014. he was elected as Assistant Professor of Surgery at the School of Medicine, University of Belgrade, and is currently employed as an attending surgeon at the Department of Esophagogastric Surgery, First Surgical University Hospital, Clinical Center of Serbia. He attended training in advanced minimally invasive surgery in Lahey Clinic, Boston, USA, and esophageal stenting at Hradec Kralovy Hospital, Czech Republic. He was a visiting professor at the Policlinico San Donato, for a one month fellowship at the University of Milan, Italy (Prof. Luigi Bonavina). In 2016. dr Skrobic attended clinical immersion course in bariatric surgery in Bucurest (Ponderas Hospital, Prof. dr Catelin Copaescu). His main clinical interests are: open and minimally invasive surgery of the foregut, pathophysiology of the foregut, surgical diagnostic and interventional endoscopy (RFA HALO system, PEG), foregut functional diagnostics (manometry, pH monitoring, impedance pH monitoring). Dr Skrobić has published more than 20 articles in scientific journals.

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