Stent treatment of perforated duodenal ulcer - physiology and clinical aspects

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To Manuel, Paulina and Inés

“Caminante, son tus huellas
el camino, y nada más;
caminante, no hay camino:
se hace camino al andar.”

Proverbios y Cantares,
Antonio Machado

Stent treatment of perforated duodenal ulcer – physiology and clinical aspects

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ABSTRACT

Background

The incidence of perforated duodenal ulcer is decreasing but still constitutes a life-threatening complication to peptic ulcer disease. Abdominal contamination from gastric or duodenal content occurs during perforations. Gastric content is normally sterile due to its low pH, but the wide-spread use of PPI might affect gastric bacterial flora. Gold standard treatment is sutured surgical closure, open or laparoscopic. Treatment with a covered stent has proven useful in cases of esophageal perforations. The same treatment strategy might be an option in selected cases with duodenal perforation. Stents placed over the pylorus might influence pyloric motility leading to stent migration. The aim of this thesis was to investigate the use of a covered stent to treat perforated duodenal ulcers including aspects on pyloric physiology and gastric bacterial colonization.

Methods

Paper I & II: Gastric and duodenal bacterial colonization was investigated taking swab samples from the mucosa for culturing during clinical outpatient gastroscopies. PPI consumption was recorded. In paper II gastric pH was measured from gastric aspirate and bacterial growth was quantified.
Paper III: Pyloric physiology was studied in an animal model using the EndoFLIP™ probe, mimicking a stent placed in the pylorus. Pyloric cross sectional area and pressure was recorded.
Paper IV: Randomized clinical trial, patients presenting with signs of upper gastrointestinal perforation and free air on a CT scan were included and randomized to surgical closure or stent treatment. Laparoscopy was performed in all patients to verify the diagnosis.

Results

Paper I: 103 patients were analyzed. Gastric and duodenal bacterial colonization was more common in patients on continuous PPI treatment ($p<0,0001$). Dominating bacterial species were of oropharyngeal origin, most common were *Streptococcus salivarius* & *mitis*.
Paper II: 107 patients were analyzed. Abundant bacterial growth ($>10^4$ CFU/ml) occurred in 16% in the stomach and 12% in the duodenum, significantly more in patients with PPI treatment ($p<0,0001$). Patients with abundant growth showed high gastric pH and old age.
Paper III: When pylorus is stepwise dilated, it changes activity from acting as an opening and closing sphincter to a propulsion pump. At full distention, pyloric motility disappears. Pyloric opening and emptying is stimulated by food.
Paper IV: 43 patients were included, 28 had a verified perforated duodenal ulcer, 15 randomized to surgical closure and 13 to stent treatment. Morbidity was 42% overall, 6 patients in each group had a complication of Clavien-Dindo grade 2-4 (n.s.). Mortality was 4% (n=1). For all patients, time from onset to intervention >12h correlated with complications Clavien-Dindo grade 3-5.

Conclusion

Bacterial flora found in the stomach and/or duodenum is mainly of oropharyngeal origin, more frequently occurring in patients with ongoing PPI treatment. Individuals with high gastric pH are more at risk for abundant gastric and/or duodenal bacterial colonization. Stent design influences pyloric motility, through pyloric distention, and seems to be of importance to avoid stent related complications. Stent treatment of perforated duodenal ulcer seems to be as safe and effective as surgical closure.

Keywords: Stent, Gastroscopy, Perforated duodenal ulcer, Proton Pump Inhibitor, Gastric bacterial flora, Gastric pH, Pylorus, EndoFLIP™, Pyloric motility.

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SAMMANFATTNING PÅ SVENSKA

Bakgrund

Brustet sår i tolvfingertarmen (perforerat ulcus duodeni) är ett livshotande tillstånd där innehåll från mag-tarm-kanalen läcker ut i bukhålan. Magsäcksinnehållet är normalt sterilt på grund av magsyrans låga pH-värde (pH <4). Vid behandling med moderna magsårläkemedel stiger pH-värdet i magsäcken, vilket gör det möjligt för bakterier som sväljs ner från munhålan till magsäcken att etablera sig. Standardbehandling av perforerat ulcus duodeni är sedan 1885 operation med förslutning av perforationen. Vid perforation i matstrupen rekommenderas idag stentbehandling med ett metallstent täck med ett silikonskikt, vilket täcker över defekten och förhindrar fortsatt läckage. Detta skapar förutsättningar för läkning. Samma princip skulle kunna användas vid perforerat sår i tolvfingertarmen. En risk vid stentbehandling är stent-glidning (migration). Syftet med denna avhandling var att studera användning av täckt stent vid perforerat ulcus duodeni. Syftet var också att studera stentets inverkan på pylorus (nedre magmunnen) fysiologi samt att kartlägga bakterieväxten i magsaften.

Metod

Delstudie I & II: Bakterieväxten kartlades i prover tagna från magsäcks-slemhinnan i samband med gastroskopi. Konsumtion av magsårsläkemedel, PPI, vilka påverkar magsäckens pH-värde noterades. I delstudie II mättes pH-värdet i magsaft som sugits upp vid gastroskopin. Bakterieväxten kvantifierades och graderades som ingen, måttlig (10^2 - 10^4 CFU/ml) eller riklig (>10^4 CFU/ml).
Delstudie III: Pylorus fysiologi; dess rörlighet, tryck och öppningsdiameter, studerades med en specialdesignad ballongformad sond (EndoFLIP™).
Delstudie IV: Randomiserad klinisk studie. Patienter som kom till akutmottagningen med symtom på perforation i mag-tarm-kanalen samt fri gas i bukhålan på datortomografi inkluderades och randomiserades till stentbehandling eller kirurgisk förslutning. Samtliga patienter genomgick laparoskopi för att bekräfta diagnosen. Postoperativt registrerades kliniskt tillfrisknande och komplikationer.

Resultat

Delstudie I: Data från 103 patienter analyserades. Bakterieväxt i magsäck och tolvfingertarm var mer vanligt hos patienter med pågående PPI-behandling ($p<0,0001$). Bakterier som förekom mest i odlingarna hade sitt ursprung i munhåla och svalg. Vanligast var *Streptococcus salivarius & mitis*.
Delstudie II: Data från 107 patienter analyserades. Riklig bakterieväxt förekom i magsäcken hos 16 % av patienterna samt i tolvfingertarmen hos 12%, signifikant oftare hos patienter med PPI-behandling ($p<0,0001$). Patienter med riklig bakterie-växt hade högre pH-värde i magsäcken och var äldre.
Delstudie III: När pylorus spänns ut förändras dess mekaniska funktion från att enbart öppna och stänga sig till att fungera som en framåtdrivande pump. Vid maximal distendering släcks dess aktivitet helt.
Delstudie IV: 43 patienter inkluderades, 28 hade ett verifierat perforerat sår i tolvfingertarmen, 15 randomiserades till kirurgisk förslutning och 13 till stentbehandling. Komplikationsfrekvensen var 42% och skiljde sig inte åt mellan grupperna, 6 patienter i vardera gruppen drabbades av en komplikation grad 2–4 enligt Clavien-Dindo-klassificeringen. Mortaliteten var 4% (1 patient). Dessa resultat motsvarar resultat från tidigare studier av perforerat sår i tolvfingertarmen. Patienter som behandlades efter >12 timmar från insjuknandet drabbades i högre grad av allvarlig komplikation, grad 3–5 enligt Clavien-Dindo, oberoende av behandlingstyp.

Konklusion

Bakterieväxten i magsäcken och tolvfingertarmen härrörde från nedsvald saliv från munhåla och svalg och förekom oftare hos individer med pågående PPI-behandling. Högt pH i magsäcken predisponerade för riklig bakterieväxt. Pylorus motilitet påverkas av ett stents design och egenskaper via dess distention av pylorus, vilket kan vara av betydelse för risken för stentglidning. Stentbehandling av perforerat ulcus duodeni tycks vara lika effektivt och säkert som kirurgisk behandling.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Arroyo Vázquez JA**, Henning C, Park PO, Bergström M. Bacterial colonization of the stomach and duodenum in a Swedish population with and without proton pump inhibitor treatment.
JGH Open. 2019 Oct 1;4(3): 405-409. PMID: 32514445

II. **Arroyo Vázquez JA**, Sjöberg M, Henning C, Bergström M, Park PO. Gastric bacterial colonization with relation to PPI consumption and gastric pH, in a Swedish population.
In **Manuscript**.

III. **Arroyo Vázquez JA**, Bergström M, Bligh S, McMahon BP, Park PO. Exploring pyloric dynamics in stenting using a distensibility technique.
Neurogastroenterol Motil. 2018 Dec; 30(12). PMID: 30109904

IV. **Arroyo Vázquez JA**, Khodakaram K, Bergström M, Park PO. Stent treatment or surgical closure for perforated duodenal ulcers: a prospective randomized study.
Surg Endosc. 2020 Nov 30. Epub ahead of print. PMID: 33258032

CONTENT

ABBREVIATIONS

H. pylori	*Helicobacter pylori*
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
PPIs	Proton Pump Inhibitors
NOTES	Natural Orifice Transluminal Endoscopic Surgery
SEMS	Self-expandable Metal Stents
CFU/mL	Colony forming units per milliliter
EndoFLIP™	Endolumenal Functional Lumen Imaging Probe
mmHg	Millimeter of mercury
ml	Milliliter
CT-scan	Computerized tomography scan
C-D	Clavien-Dindo classification
CRP	C-reactive protein
WBC	White blood cells

DEFINITIONS IN SHORT

ASA-score	American Society of Anaesthesiologist score for physical status classification assessing fitness of patients before surgery
C-D	Clavien-Dindo classification, grading system of postoperative complications

1 INTRODUCTION

1.1 PEPTIC ULCER

1.1.1 HISTORICAL BACKGROUND OF PEPTIC ULCER DISEASE

Peptic ulcer perforations have been described since the 17th century. There are historical descriptions of individuals presenting with acute abdominal pain, nausea and vomiting followed by further deterioration and death in some hours or days. This clinical picture was wrongly explained to be caused by poisoning, despite the finding of a hole in the stomach or duodenum at necropsy [1]. The daughter of King Charles I of England, Henriette-Anne was 26 years old when she died in 1670 after a period of abdominal pain and tenderness, the necropsy revealed a small hole in the stomach and peritonitis. The doctors performing the autopsy blamed the perforation on accidental puncture by instruments used during necropsy [1].

In the 18th and early 19th century patients presenting with upper abdominal pain or discomfort where usually diagnosed as dyspepsia, indigestion or gastralgia [2].

Peptic ulcer increased as a diagnosis in western countries at the end of the 19th century. Hospital records from London and New York have shown that the earliest recorded admission for gastric ulcer were in the 1840s, increasing rapidly to a maximum around 1910 to then decline [2]. According to Baron, duodenal ulcer was described at autopsy at the Middlesex Hospital during the 1850s. Admissions for duodenal ulcer were recorded in London and New York during the 1860s, followed by a rapid increase, reaching a maximum, recorded in London during the 1950s [2].

The diagnosis of ulcer has been essentially clinical, based on clinical histories. From 1890s, when surgery for peptic ulcers increased, more definite diagnoses were possible. Contrast radiology of peptic ulcer emerged during the 1920s and endoscopy became available in the 1970s [2].

At the end of the 1800s, hyperchlorhydria was recognized as a cause of peptic ulcer and treatment was directed towards control of gastric acid secretion [3]. “No acid, no ulcer” was first declared by Dragutin (Carl) Schwarz (1868-1917) in 1910, describing the role of gastric acid in the pathogenesis of peptic ulcer disease [4]. Since then, ulcer treatment has focused on acid reduction such as gastric resection, vagotomy and pharmacological treatment.

Non-surgical treatment included diet modification, as described by Bertram Welton Sippy (1866-1924). He recommended a diet based on milk, cream, eggs, cereals and vegetable purées. The hypothesis was to protect the ulcer from further gastric juice corrosion and thus obtain ulcer healing (Sippy´s therapy) [5, 6]. Diet control alone was seldom enough and surgery with gastric resection and truncal vagotomy was often necessary [3]. Peptic ulcer disease has evolved over time from an unknown condition to a surgical condition [7].

Pharmacological treatment changed from the use of diet and antacids, such as sodium bicarbonate, to the use of anticholinergics reducing acid gastric secretion, with the disadvantage of several side effects. In 1976, the first H_2-receptor antagonist, cimetidine, was introduced on the market, improving pharmacological treatment. It’s effect was considered as good as surgical vagotomy [3].

Proton pump inhibitors (PPIs) were developed during the 80s, exerting a new mechanism inhibiting HCl production in the gastric parietal cells and eventually revolutionizing the treatment of peptic ulcer disease. Omeprazole was the first PPI in clinical use in 1989 [8], followed by several similar substances. The use of PPIs has since then become standard treatment and consumption is still rising worldwide.

Helicobacter pylori were identified in 1982 by Marshall and Warren [9]. This gram-negative microaerophilic bacteria colonizes stomach mucosa, creating a local inflammation decreasing antral somatostatin production and eventually leading to increased gastrin secretion and acid production [8, 10]. Nowadays the most common causes of duodenal ulcer are described as *Helicobacter pylori* infection and intake of Non-Steroidal Anti Inflammatory drugs (NSAIDs) [11, 12]. But still, the axiom “No acid, no ulcer” prevails.

1.1.2 PEPTIC ULCER PERFORATIONS

The incidence of uncomplicated peptic ulcer disease has fallen during the last decades and the incidence of perforated peptic ulcer is also decreasing [13]. Still, a perforated ulcer constitutes a serious condition with high morbidity and mortality rates varying between 10 to 40% [14, 15]. Thorsen described in 2013 an incidence for perforated peptic ulcer in Norway of about 6,5/100 000 per year, with 10 times higher incidence for patients over 60 years. Perforated duodenal ulcers constitute approximately one third of all perforated peptic ulcers [16]. They also showed that the 30-day-mortality for patients with perforated peptic ulcer is as high as 16%, and 23% for patients with perforated duodenal ulcers. Both morbidity and mortality increase in elderly and co-morbid patients [11, 14].

1.1.3 SURGICAL TREATMENT OF PERFORATED PEPTIC ULCER

The traditional surgical treatment for perforated duodenal ulcer is sutured closure, performed with open or laparoscopic technique. Johan Mikulicz-Radecki (1850-1905) is known as the first surgeon to perform a sutured closure of a perforated gastric ulcer in 1885 [15]. Open surgical closure of the perforation can be performed with or without omentoplasty [15]. Roscoe Graham (1890-1948) describe in 1937 a technique to repair perforated peptic ulcer with surgical closure with an omental patch covering the suture site [17], a technique still in use. Laparoscopic closure for perforated peptic ulcer has been performed since the 1990s [15], and has been shown to be as safe and effective as open repair with less postoperative pain and less wound complications [18].

The risk of postoperative morbidity after surgical closure increase with high age, comorbidity, preoperative deterioration and complicated surgery with long operation time [19, 20]. Kim et al described 17% postoperative pulmonary complications, 17% wound complications and 7% multi-organ failure, in a series with 142 patients operated for perforated peptic ulcer between 2005 and 2010 [19].

Severely ill patients with high surgical risks, are sometimes treated conservatively with nasogastric tube and antibiotics, a treatment described as Taylor's method. Conservative treatment is associated with high mortality rate. Alizadeh described high mortality in a retrospective study of 332 patients with perforated ulcers, where 12 patients were treated conservatively, eight out of these 12 patients died (2/3) [21]. Saber described slightly better results in a study performed in 2012. Patients not fit for surgery were treated conservatively together with a percutaneously placed drainage of the abdominal cavity, resulting in a mortality of (20%) [22].

However, not much has changed regarding surgical treatment of perforated ulcer since Mikulicz-Radecki described it in 1885. Sutured closure is still the gold standard treatment.

1.2 STENT

1.2.1 HISTORY, USAGE AND MATERIAL

The word "Stent" originates from the English dentist Charles Thomas Stent (1807-1885) who in the 1850s invented a modelling compound to get dental impressions. He modified the gum of a Malayan tree, gutta-percha, that was used in the 19th century as a denture base by adding stearine, a glyceride of stearic, palmitic and oleic acids, and talc as a filler. This compound became known as "Stent´s compound". During the first World War a Dutch plastic surgeon Johannes Fredericus Esser (1877-1946) started using Stent´s Compound for the fixation of skin grafts in wounded soldiers, this principle was named "stenting" and it was used for facial and oral reconstructions [23, 24, 25].

The principle of stenting was further used in other areas such biliary surgery where inert tubes and biologic tissue were used to bridge an opening or replace the continuity of the bile duct.

Re Mine used a polyethylene tube to act as a stent for the anastomosis, while experimenting with biliary reconstruction in dogs in 1945. He used a skin graft

as a tube and in order to prevent contraction of the graft he applied Stent´s dressings principle [25, 26].

Dotter described in 1969 an animal model where he could open a narrowed or occluded arterial lumen in dogs, percutaneously placing a plastic tubular endovascular prosthesis. In order to improve patency and prevent the tubular prosthesis to clot, he used an open-centered coil spring of stainless steel wire. The open coil spring configuration showed long-term patency and also carried the advantage of avoiding the trauma of a surgical vascular reconstruction, replacing it with a percutaneous technique [27].

In the late 70s Pereiras described how a malignant obstruction of the biliary tree safely and effectively could be relieved by percutaneous placement of a permanent prosthesis bridging the stricture [28]. During the early 80s, Hans Wallsten designed a self-expanding metal meshwork tube in a stainless-steel alloy, the first modern metal stent, also called the "Wallstent™". This stent was initially applied in arteries in a canine model, and first placed in a human coronary artery in 1986. Clinical results were published in 1987 [29, 30]. Cragg and Dotter started later in the 80s using Nitinol wire coil stents for restoration of internal flow in vessels and biliary ducts [31, 32].

Nitinol is now the dominating material for fabrication of self-expandable metal stents. NiTiNOL is a Nickel-Titanium alloy developed in 1959 by William J. Buehler of the U.S. Navy (Ni-Ti-Naval Ordnance Laboratory) [33]. Nitinol is a metal alloy with thermal memory, a property that allows stents to be manufactured in a specific shape, then manually elongated and inserted into a delivery system, followed by recovery of the original shape when released inside the body, thus exerting radial force on a stricture [34]. Self-Expandable Metal Stents (SEMS) are now widely used in the gastrointestinal tract.

1.2.2 ENDOSCOPIC STENT TREATMENT OF GASTROINTESTINAL STRICTURES AND PERFORATIONS

Rigid plastic tubes were used by Symonds, as early as in 1885, to relieve dysphagia caused by malignant esophageal strictures [35], but due to high complication rates their use declined. Metallic stents were later developed for use in the esophagus, for palliation of malignant dysphagia, and showed better

outcome [36, 37]. Frimberger described in 1983 the use of an expanding spiral made of metal for palliation of malignant esophageal dysphagia assuming less risk of perforation than with conventional tubes [38]. Metal stents were also used for managing lesions in the stomach, duodenum and colon [39]. In 1993 Song reported the use of a covered metal stent implanted through a surgical gastrotomy, to relieve obstruction from an antral carcinoma [40]. Strecker reported in 1995 the use of a self-expanding nitinol stent in a duodenal stenosis with an oral approach [41]. The use of stents to relieve large bowel obstruction was first reported by Dohmoto in 1991, when it was used for palliation of malignant strictures [39, 42].

Endoscopic stent treatment of malignant fistulas and perforations in the esophagus was first tried out during the 90s, using a plastic-covered metallic stent [43]. Y S Do described in 1993 the use of a self-expanding silicone-covered tube for palliative treatment of esophago-respiratory fistulas in patients with esophageal carcinoma [44]. In 1995 Watkinson described the use of plastic-covered self-expanding metallic endo-prosthesis for treating patients with perforation in the esophagus, caused during dilatation of malignant obstructions [45].

Treatment of esophageal perforations, iatrogenic or spontaneous, with a covered self-expandable metal stent together with percutaneous drainage of the pleural cavity is currently considered to be standard treatment. This regime has shown good results and has lowered mortality [46, 47, 48].

The same method, placement of a covered stent together with drainage, is currently used to treat anastomotic leakage after gastric-bypass surgery [49, 50] also with good results. The main advantage of stent treatment in these patients is the avoidance of major surgery, possibly decreasing morbidity and mortality.

In analogy with the described techniques to treat perforations with a covered stent and drainage, we started, in 2008, to treat selected patients with perforated duodenal ulcers with covered stents and drainage [51]. The first two patients in this series were treated with stent due to leakage after primary surgery. Subsequent patients were treated with duodenal stent because of high comorbidity or high surgical risk. In this series, the mortality was 1/8. The patients could start oral intake after a median of 3 days (0-7). Median hospital stay was 17 days (9-36).

Different endoscopic methods have been tried to treat perforations of the gastrointestinal tract. Hashiba described in 2001 an experimental method for

endoscopic repair of gastric perforations with an omental patch in an animal model. In this study, a perforation of the anterior stomach wall was sealed endoscopically with an omental patch that was pulled into the perforation and fixated [52]. Endoscopic treatment for perforated peptic ulcer have been performed lately using the "over the scope clips" (OTSC), published as a case report [53]. This method would be difficult to use in perforated duodenal ulcers due to the lack of space in the duodenum and because of fibrotic changes of the tissue around the perforation site.

Different techniques for endoscopic stitching and suturing were developed and tried out during the evolution of Natural Orifice Transluminal Endoscopic Surgery (NOTES) in early 2000 [54]. T-tag-based techniques allowing for stitching through a gastroscope were developed, for example the tissue apposition system (TAS) [55]. This technique was clinically used for sutured closure of both anastomotic leakage and perforated duodenal ulcer [56]. However, T-tag-suturing has not been further developed and is not commercially available today. Endoscopic suturing in the gastrointestinal tract has evolved and nowadays, the OverStitch endoscopic suturing system, is clinically used for example in closure of endoscopic perforations, stent fixation, fistula or leak closure, bariatric surgery, etc [57, 58]. Due to the size of the device it is difficult to use in the narrow space of the duodenum for closure of an ulcer perforation [59].

1.3 DUODENAL STENT TREATMENT AND MIGRATION

A major concern using stents to cover leakage after surgical closure or a perforation, is stent migration. For stent treatment of a perforation, a stent covered with a polyurethane coat is used and migration can occur either backwards up into the stomach or downwards into the small bowel. Downward migration constitutes a serious complication, often requiring surgery. Covered stents do not attach to the bowel mucosa in the same way as uncovered stents. Uncovered stents show lower migration rates, but cannot be used for sealing of perforations [60]. When treating a leakage, the stent is not placed over a stricture that can help keeping it in place, also increasing the risk of migration.

Stent design and technical properties are believed to influence the risk of migration [61]. Stent manufacturers have tried different designs to reduce this risk, but there is no data published in scientific papers. Despite the wide use of stent treatment for different medical conditions there are hardly any publications or studies describing how stents affect intestinal motility. Retrograde duodenal motility might be one reason for stent migration backwards into the stomach [62]. The pylorus is believed to be a sphincter, opening in response to stimuli from the content in the antrum and duodenum [63], this response might also affect a stent placed over the pylorus. When treating patients with perforated duodenal ulcers with covered stents, the stent is placed through the pylorus and down into the proximal part of the duodenum, which might provoke increased intestinal motility.

The geometrical shape of a stent and its radial force and stiffness are believed to affect its propensity for migration. Various stent characteristics may influence stent behavior in different ways when applied in different locations of the gastrointestinal tract, but this has not yet been scientifically studied. It is difficult to study stent physiology in vivo, why stent development and improvements have been based on empirical data and clinical outcomes.

EndoFLIP™ is a device for assessing gastrointestinal sphincters by measurement of sphincter cross-sectional area/estimated diameter and pressure. The probe consists of a balloon that can be step-wise inflated with saline and measurements are simultaneously demonstrated on a display, giving a visual image of the sphincter estimated diameter at up to 16 locations, 5 mms apart, along with the pressure inside the bag-like balloon. In the current experiment, we placed the balloon-probe inside the pylorus, filling it to different distentions to mimic the pyloric provocation caused by a stent.

1.4 GASTRIC BACTERIAL FLORA

The microbial flora in the gastrointestinal tract is dynamic. There are 500 to 1000 different bacterial species in the gastrointestinal tract [64]. The prevalence of bacteria in the different parts of the gastrointestinal system is influenced by age, genotype, diet and medication, for example PPIs. Studies suggest that the bacterial flora differs between the oral cavity, esophagus and stomach as compared with the small and large intestines [64, 65].

One of the physiologic roles of the stomach is to disinfect whatever is swallowed before it continues down the small intestine. HCl is a strong acid and in humans the normal gastric pH is about 2 [8]. Low pH is an important factor of the "gastric bactericidal barrier". Besides *Helicobacter pylori*, few bacteria can grow in the stomach due to the acidic conditions. A reduction of the gastric acid secretion, induced by medication (H_2-blockers or PPIs) or atrophic gastritis, leads to hypochlorhydria (pH>4 and <7) or achlorhydria (pH>7) increasing the susceptibility for bacterial overgrowth [66, 67]. It has been shown that a gastric pH above 4 allows bacterial colonization of the stomach [68].

The gastric flora may be influenced by the widespread use of PPIs of today. According to the National Board of Health and Welfare in Sweden, the consumption of PPI during the period of 2006-2016 in Sweden, measured in daily doses per 1000 inhabitants, showed an 88% increase [69]. Ongoing treatment with PPIs leads to increasing gastric pH levels, facilitating bacterial overgrowth in the stomach [70]. Rosen et al found a significant difference in gastric bacterial colonization in pediatric patients with and without PPI treatment [71].

Bacterial contamination of the abdominal cavity is a serious concern and could be an issue during gastric surgery, transgastric endoscopic interventions or perforations of the upper gastrointestinal tract. Perforated peptic ulcers are believed to resemble clean-contaminated cases in the acute phase due to the low pH making the gastric content sterile.

In a study, rats were preoperatively treated with PPIs and then operated with stomach exposure. Aspiration of the gastric content was injected into the peritoneal cavity to mimic gastric spillage during transgastric surgery. This experiment resulted in an increased risk for bacterial colonization of the peritoneal cavity and development of intra-abdominal abscess [72]. Bacterial

contamination of the abdominal cavity in patients with ongoing PPI treatment has been described in a study with patients undergoing laparoscopic gastric-bypass surgery, but without postoperative abscess formation [73]. The period of abdominal exposure for gastric content may be of importance. Long time contamination of the abdominal cavity in patients with perforated duodenal ulcer has been shown to affect morbidity and mortality [11]. In order to refine the selection of antibiotic treatment, further characterization of the gastric flora is needed. Today this is increasingly important due to the broad and common use of PPIs.

HYPOTHESIS

Stent treatment constitutes a possible treatment alternative in perforated duodenal ulcer

The hypothesis was divided as follows:

- Todays increased intake of PPIs influences the bacterial flora of the stomach.

- A stent placed over the pylorus may influence pyloric motility.

- Stent treatment together with percutaneous drainage of the peritoneal cavity is a safe and effective treatment for perforated duodenal ulcer.

AIMS

I. To study bacterial flora in the stomach in patients referred for gastroscopy, with and without ongoing PPI treatment.

II. To quantify gastric and duodenal bacterial growth in relation to gastric pH and PPI intake.

III. To study how a stent affects pyloric physiology, especially motility.

IV. To compare outcome between surgical closure and stent treatment for perforated duodenal ulcer in a prospective randomized multicenter study.

2 METHODS

2.1 BACTERIAL COLONIZATION OF THE STOMACH, PAPER I & II

2.1.1 GASTROSCOPY

Patients referred to the endoscopy unit at the South Älvsborg Hospital for gastroscopy, were approached and assessed for eligibility. Exclusion criteria were: age below 18 years, antibiotic intake within 3 months prior to gastroscopy, known altered gastric anatomy, on-demand PPI intake, ongoing immune-modifying treatment, need for language translation and non-autonomous patient unable to give consent. Patients were also excluded if a suspected malignancy was found during the gastroscopy.

Local routines of the endoscopic unit were followed, no intake of solid food or liquids for 6 h prior to the gastroscopy. The gastroscopy was performed with the patients in the left lateral decubitus position. An endoscopically experienced surgeon performed all the gastroscopies.

2.1.2 SAMPLING PROCEDURE FOR pH MEASUREMENT (PAPER II)

No foam-dissolving agent, such as simethicone, was given prior to the examination. The gastroscope was introduced into the stomach without using suction. The first gastric liquid that was found was aspirated and collected using a suction-trap. Gastric pH was then determined using a calibrated pH-meter (Voltcraft PH-100 ATC, range 0-14 pH, accuracy ± 0,07 pH).

2.1.3 SAMPLING PROCEDURE FOR BACTERIAL CULTURING (PAPERS I & II)

Gastric sampling for bacterial culturing was performed using a standard covered cytology brush (Fig. 1). The brush was located inside its plastic cover when brought down the working channel, it was then exposed for rubbing the mucosa and finally retracted inside the plastic cover before being brought up. Care was taken to minimize contamination. The tip of the brush was cut off into a sterile tube containing 0,9% saline. The same procedure was then repeated for sampling in the duodenal bulb using a new cytology brush. Duodenal sampling was performed for patients no 64-114 in study I and for all patients in study II.

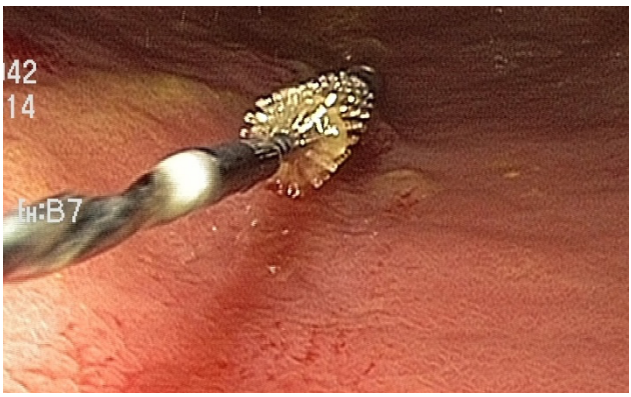

Figure 1. Single-use cytology brush, rubbing the gastric mucosa.

2.1.4 MICROBIOLOGICAL PROCEDURES (PAPER I)

All samples were brought to the hospital laboratory for microbiology and were cultured within 4 h. Culture was performed on GC agar (GC agar-acumedia with 2% hemoglobin and 1% isovitalex), blood agar (Blood agar base no 2-Oxoid with 5% horse blood) (Fig. 2), and anaerobic agar with two 10 µg gentamicin discs (Fastidious anaerobic agar-acumedia with 4% human blood).

GC agar was incubated in 6% CO_2, blood agar in normal air, and anaerobic agar in an anaerobic box (N_2 with 10% H_2 and 10% CO_2) at 36°C for 48 h. GC agar and blood agar were inspected daily and anaerobic agar after 2 days. Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) (VITEK-MS™, Biomérieux) was used to identify different bacterial colonies.

The samples collected from the antrum of the stomach were cultured and analyzed for common Gram-negative bacteria (*Escherichia coli, Klebsiella, Enterobacter,* Proteus, Pseudomonas) and Gram-positive bacteria (*Staphylococcus, Streptococcus*), common bacteria of the oral cavity (alpha-streptococci, *Neisseria, Haemophilus,* pneumococci), and anaerobes (*Bacteriodes, clostridia*). The cut-off value for the cultures was 50 CFU/ml.

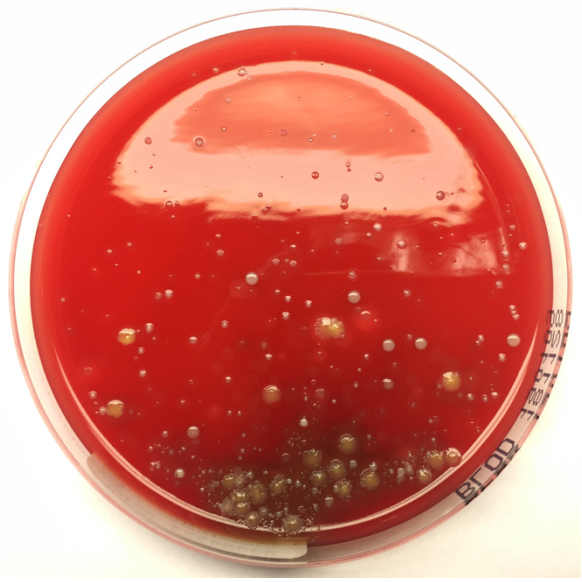

Figure 2. Blood agar cultivation plate.

2.1.5 MICROBIOLOGICAL PROCEDURES INCLUDING QUANTIFICATION OF BACTERIAL GROWTH (PAPER II)

All samples were cultured within 4 h. Culture was initially performed as in paper I. Blood and GC-agar plates were assessed after one and two days and on day two, colonies were distinguished by macroscopic morphology. A growth of CFU $\geq 10^4$ CFU/ml of a specific colony type in the primary sample tubes was considered abundant growth. This corresponds to 200 CFU per agar plate from original samples, and 4 CFU per agar plate from the diluted samples. This level was considered the cut off-levels for further analysis. The cut off for diluted samples was selected with the assumption that CFUs occurred by Poisson distribution of events rather than Gaussian distribution. To reduce the risk of random results, the lower 2.5th percentile of a 95 % Poisson confidence interval should be $\geq 10^4$ CFU/ml, which is achieved by selecting 4 CFU as a cut off. Abundant bacterial colonies were isolated onto new agar plates and incubated as described above, for at least one day.

Abundant bacterial colonies were typed to species or group level with mass spectrometry using MALDI-TOF (VITEK-MS^{TM}, Biomérieux).

2.1.6 H. PYLORI DETECTION (PAPER I)

Paper I: Helicobacter pylori was detected using a urease test (HelicotecUT Plus, Strong-biotech Corporation) on biopsy specimens obtained from the antrum of the stomach.

2.1.7 CONTROL-SAMPLING FROM GASTROSCOPES (PAPER II)

The gastroscopes (Olympus GIF HQ190) were handled according to routine and cleaned in standard endoscope-dishwashers (Olympus ETD3). The outcome of standard cleaning is not considered fully sterile and therefore all

gastroscopes (n=13) at the Endoscopy Unit were controlled for microbial growth post cleaning. This was done using an ESwab® (Copan Italia) that was swabbed thoroughly in and around the distal orifice of the working channel. Prior to gastroscopy, endoscopes (n=5) were flushed through the working channel with 5 ml of sterile saline solution that was collected in a sterile test tube. All samples were handled and processed as above for culturing.

2.2 PYLORIC DYNAMICS USING A DISTENSIBILITY TECHNIQUE, PAPER III

2.2.1 ANIMAL MODEL AND PROCEDURE

Five female Swedish Landrace pigs (28-30 kg) were used in a non-survival model. The animals were kept on a liquid diet for 3 days before the study and were allowed only water (free access) from 12 hours before the procedure.

All procedures were performed under a standard general anesthesia protocol executed by veterinary staff. The EndoFLIP™ catheter was used through a gastroscope for testing of pyloric distensibility, mimicking stent-treatment. Gastroscopy was performed using a 6-mm accessory channel gastroscope (Model GIF-XTQ160; Olympus Corporation, Tokyo, Japan), and the pylorus was visualized. EndoFLIP™ catheter model EF 353 (Crospon, Galway, Irland) was used. A modification to the distal tip of the catheter allowed a guide wire (Jagwire; Boston Scientific, Marlborough, MA, USA) to be fitted through a small hole at the tip without damaging the catheter. The guidewire was inserted into the working channel of the gastroscope and directed visually through the pylorus and into the duodenum. The EndoFLIP™ catheter (Fig. 3) was fitted over the wire and brought down through the endoscope channel and out through the pylorus. The probe was placed straddling the pyloric sphincter and its position was confirmed by filling 20 ml of saline solution into the balloon and observing the classic hourglass shape on the EndoFLIP™ system screen (Fig. 4). The optimal position of the probe was considered to be when the central measurements represented the narrowest region in the sphincter.

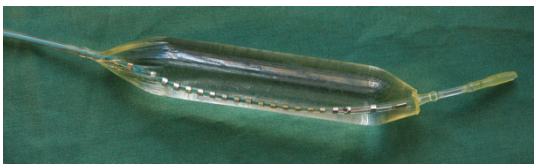

Figure 3. EndoFLIP™ catheter.

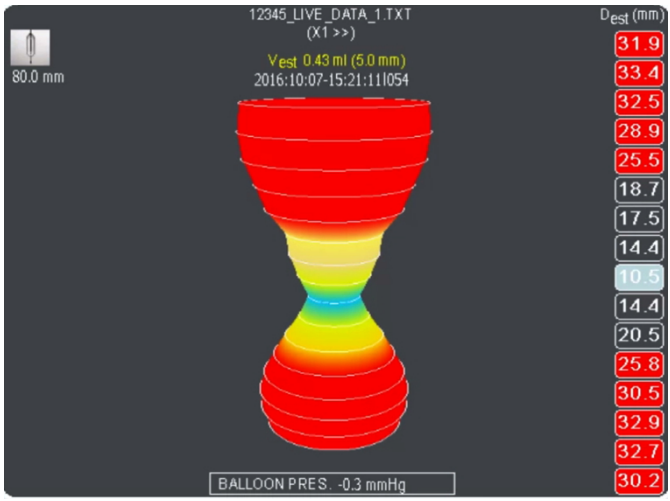

Figure 4. Hourglass shape on the EndoFLIP™ system screen.

2.2.2 ANIMAL STUDY DESIGN

In the first pig studied, baseline distensibility measurements and prokinetic drug test were performed followed by placement of a duodenal stent. Measurements were repeated inside the stent. In subsequent pigs, baseline distensibility test, a prokinetic test and a meal test were performed. Between each test, a period of 1 hour was allowed to let the pylorus recover.

2.2.3 BASELINE DISTENSIBILITY TEST – ALL ANIMALS

The EndoFLIP™ probe was deflated until all the liquid was removed and the pressure was set to 0 mmHG as per operator protocols. First distention was performed filling the balloon with 20 ml of saline; these readings were considered to be the physiologic baseline of the pylorus. Standard protocol

followed with two continuous ramp distension to 50 ml followed by two series of stepwise distensions at 20, 30, 40, and 50 ml, respectively, stopping for 20 seconds at each step. The start time for all events was recorded and used to recall the data from the study file. The test protocol is illustrated in Figure 5.

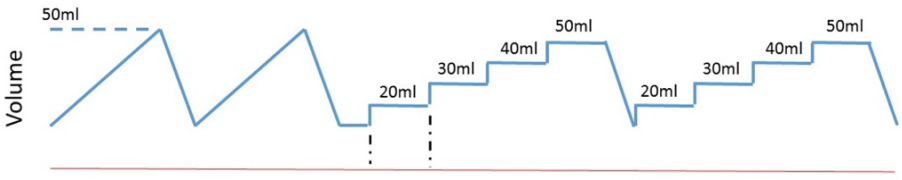

Figure 5. Test protocol for distention.

2.2.4 STENT TEST – ONE ANIMAL

In the first pig, a partially covered stent (Hanaro, 9 cm duodenal stent NCN; MI-tech, Pyeongtaek, Korea) was placed over a guidewire, through the scope. The stent was placed with the proximal end in the antrum and the distal end in the duodenum. The EndoFLIP™ catheter was fitted over a guide wire and brought down through the endoscope channel and placed inside the stent. Measurements were performed following the standard test protocol.

2.2.5 PROKINETIC TEST – FIVE ANIMALS

In five pigs, neostigmine was administrated intravenously at a dose of 0.036 mg/kg, and after a wait of 5 minutes, the stepwise protocols were repeated. After 30 minutes, a distension test identified that the effects of the neostigmine were no longer apparent and a further set of stepwise volume controlled distensions were repeated.

2.2.6 LIQUID MEAL TEST – FOUR ANIMALS

A feeding tube was introduced into the pig stomach. A mixture of 300 ml feeding formula and 150 ml of water was instilled. The probe, still placed in the pylorus, was then infused using the previously described distension protocol. The test was also repeated after a 30-minute wait.

2.2.7 PILOT HUMAN STUDY

Gastroscopy was performed in conscious sedation, using the large channel (Fig. 6). The above-described technique was used for inserting the EndoFLIP™. A baseline distension test was performed followed by motility stimulation using metoclopramide (10 mg iv) and a new distension test was performed after 5 minutes.

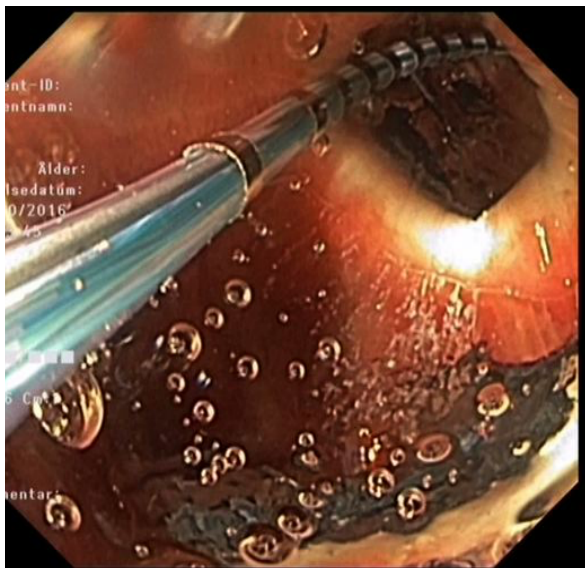

Figure 6. EndoFLIP™ placed in human pylorus.

2.3 STENT TREATMENT OR SURGICAL CLOSURE FOR PERFORATED DUODENAL ULCERS, PAPER IV

2.3.1 DESIGN

A multicenter randomized control trial started at five regional hospitals in the Region of Västra Götaland, Sweden, in order to increase the number of included patients.
Patients presenting at the emergency room with abdominal pain, clinical signs of a perforation of the upper gastrointestinal tract and free abdominal air on a CT-scan were approached for inclusion.
Information about the study and informed consent was achieved by the surgeon on call.
Inclusion was performed between December 2014 and August 2018. Non-surgical candidates or patients in critical condition unable to sign the consent were not included. Patients under 18 years and patients in need of a translator were not approached for inclusion (Fig. 7).

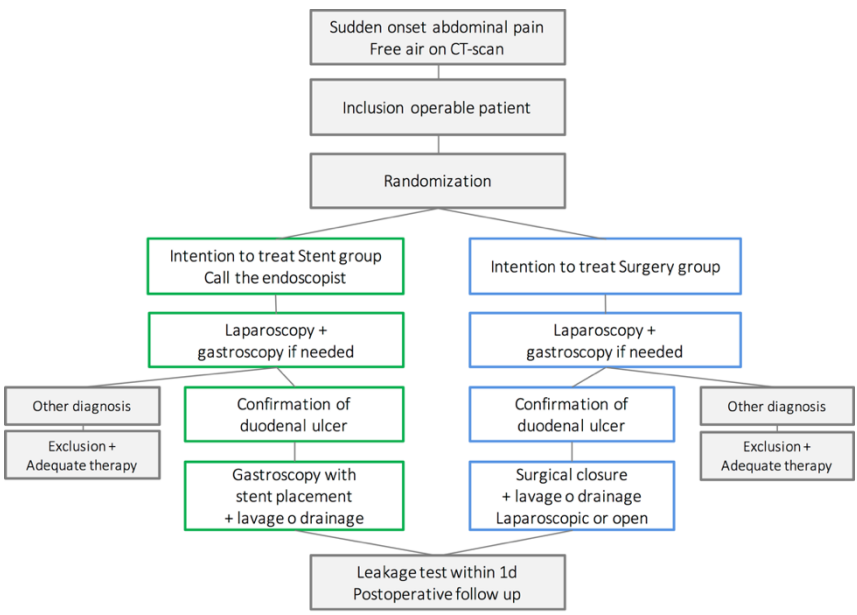

Figure 7. Flow chart, study protocol.

2.3.2 RANDOMIZATION

Patients were randomized to surgical closure or stent treatment after inclusion. Randomization was done by allocation of patients in a 1:1 ratio in balanced blocks of six (three of each). Four envelopes were used out of each block.

2.3.3 INTERVENTIONS AND FOLLOW UP

Laparoscopy was performed in all patients to establish the diagnosis and to perform lavage. If needed a peroperative gastroscopy was done to verify the presence of a perforated duodenal ulcer. Patients were treated according to the assigned group. Surgical closure was performed with open or laparoscopic techniques according to the surgeon´s preference. In patients randomized to stent treatment a per-operative gastroscopy was performed using a therapeutic gastroscope (Model GIF-2TH180; Olympus Corporation, Tokyo, Japan), to place the stent through the gastroscope. The gastroscope was passed beyond the place of perforation, a guide wire was placed through the scope into the proximal part of the jejunum and a partially covered duodenal stent (Hanaro, MI-tech Korea) (Fig. 8) was advanced and released over the guide-wire (JAGwire; Boston Scientific, Marlborough, M, USA) to cover the perforation. The oral end of the stent was placed above the pylorus and the covered part of the stent at the perforation site.

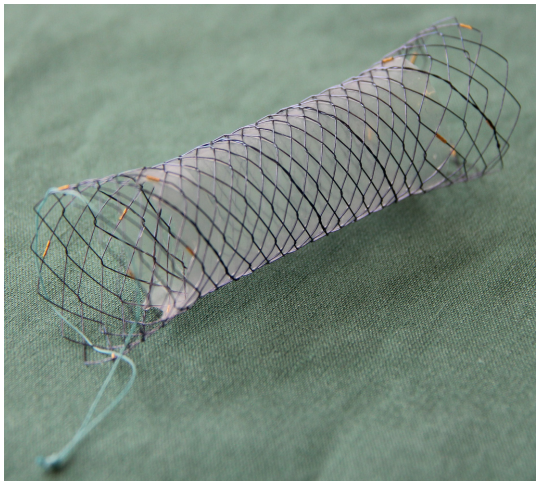

Figure 8. Partially covered duodenal stent from Hanaro, MI-tech, Korea.

Lavage of the abdominal cavity was performed using warm saline in all patients and an abdominal passive 20 Fr drain was placed at the site of the perforation. All patients were treated with broad spectrum antibiotics (Piperacillin-Tazobactam 4g/0.5g three times a day) and intravenous PPI (Pantoprazol 40mg two times a day) until oral intake was allowed.

On post-operative day one, a methylene blue test was performed in all patients (250 ml of water mixed with 5 ml methylene blue given orally). If blue color was observed in the abdominal drain, the patient was further evaluated for a salvation stent treatment in the case of a patient randomized to surgical closure, or a new stent placement in the case of a patient previously randomized to stent treatment.

If there were no observed signs of leakage, the patient was allowed oral intake of liquids during the first post-operative day, increasing to soft food after a couple of days for patients randomized to surgical closure.

To decrease the risk of stent migration, patients randomized to stent treatment were only allowed liquid diet until the stent was removed. A nutritionist monitored postoperative oral intake and nutrition, the daily need of calories was calculated for each patient in both groups. If needed, supplementary parenteral nutrition was given. Liquid diet was adjusted to be as nutritious as ordinary diet.

Complications were treated according to local guidelines.

Stents were endoscopically removed 2-3 weeks after placement and the perforation site was inspected. If signs of remaining perforation where suspected a new stent was placed for two more weeks.

Demographic data, ASA-score, operation time, complications according to the Clavien-Dindo grading system [74], and hospital stay were recorded. Blood levels of CRP and WBC were followed at least 3 days post-operatively.

2.4 STATISTICS

Non-parametric statistical methods were utilized for most of the analyses. The Wilcoxon signed-rank test was used for related data and the Mann-Whitney *U* test for nonrelated data. The χ^2 test was used for nominal data and the Kruskal-Wallis test for multiple comparisons. Values are given as median and range in most of the papers. In paper III, values are given as means with 95% confidence intervals. Differences were considered statistically significant at $p < 0.05$. All data processing was performed using the IBM SPSS statistics software.

2.5 ETHICS

Paper I

The study was approved by the Regional Ethics Committee of Västra Götaland, Sweden (Dnr 054-11) and registered in Researchweb, trial registration number 98041.

Paper II

The study was approved by the Regional Ethics Committee of Västra Götaland, Sweden (Dnr 910-17) and registered in Researchweb.

Paper III

The animal study was approved by the Gothenburg animals ethics committee (133-2015).

Paper IV

The study was approved by the Regional Ethics Committee of Västra götaland, Sweden (Dnr 527-14) and registered in Researchweb with trial registration number 144881.

3 RESULTS

3.1 PAPER I

3.1.1 DEMOGRAPHICS

A total of 114 patients were consented for the study and after exclusions 103 individuals were eligible for data analysis. Reasons for exclusions were: missed recent intake of antibiotics, on-demand use of PPI and unanticipated altered anatomy. The most common indications for gastroscopy were reflux symptoms (30%), nausea and/or vomiting (19%), abdominal pain (18%), bleeding or anemia (15%).

Out of the 103 patients, 53 had continuous PPI treatment and 50 had no PPI treatment. Median age was significantly higher among patients with PPI treatment (62 years (20-89)) compared with those without (45 years (20-82) p=0,02). There was no difference in gender distribution between PPI-users and non-PPI-users (overall 59% female).

3.1.2 CULTURES

A positive gastric culture was found in 55/103 patients, and a positive duodenal culture was found in 28/49 patients with parallel duodenal sampling. Positive culture in both stomach and duodenum was found in 21 patients. Significantly more patients with PPI treatment had a positive gastric culture (79%, 42/53), compared with 26% (13/50) among the non-PPI users ($p<0,0001$). A similar difference was recorded for duodenal samples, 83% (20/24 PPI users) vs 32% (8/25 non-PPI users) ($p<0,0001$) (Fig. 9).

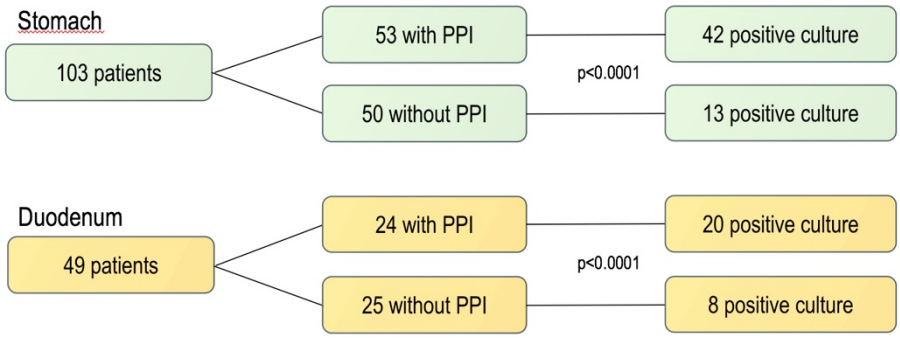

Figure 9. Included patients, PPI usage and bacterial cultures.

Age did not differ between patients with positive and negative cultures among non-PPI consumers. Patients with PPI treatment and positive gastric culture were significantly older than those with PPI treatment and a negative gastric culture (p=0,001) (Fig. 10).

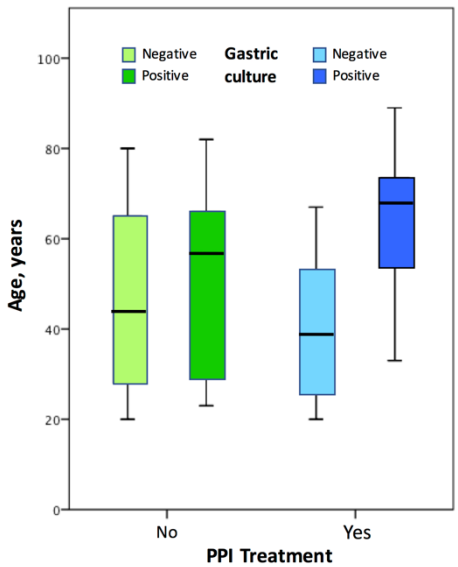

Figure 10. Age, PPI and gastric culture.

Streptococcal strains of several oral subtypes were the most commonly identified bacterial species. The dominating strains were *Streptococcus salivarius* and *Streptococcus mitis*, followed by *Neisseria* species (*mucosa/subflava*) and *Streptococcus parasanguinis* and *Staphylococcus epidermidis*, *Staphylococcus capiti, Staphylococcus aureus, Rothia mucilaginosa, Lactobacilus (catharralis, ghassesi),* alpha-streptococci, *Streptococcus vestibularis* and *E. coli* (Fig. 11).

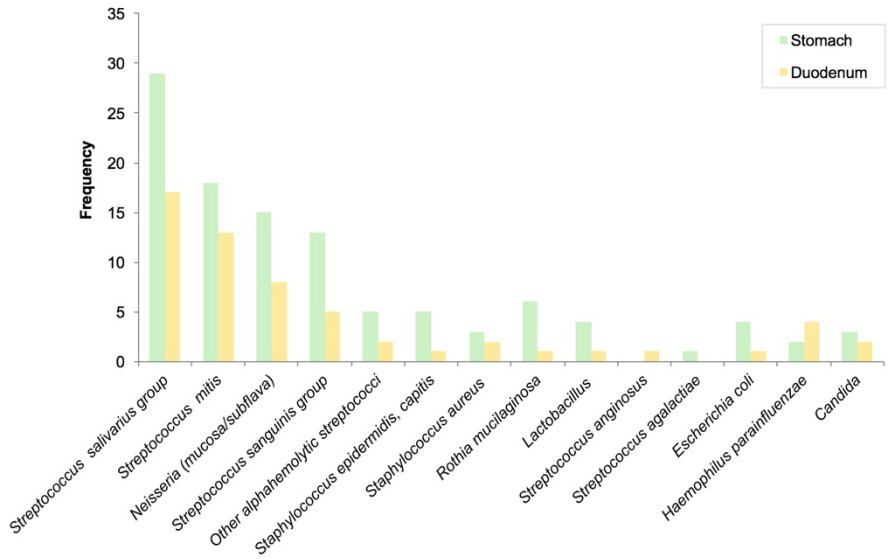

Figure 11. Bacterial species found in the stomach and duodenum.

Most patients with a positive gastric and/or duodenal culture (36/55) were colonized with more than one strain of bacteria (2-7).

3.1.3 H. PYLORI

Ten out of the 103 patients had a positive urease test, indicating presence of *H. pylori,* 3 women and 7 men (n.s.). Nine of the patients with positive urease test were not treated with PPI.

3.2 PAPER II

3.2.1 DEMOGRAPHICS

A total of 118 patients were included and consented for sampling and pH measurement. Eleven patients were excluded due to findings of malignancy during the examination or protocol violation concerning procedure or sample management. After exclusions, 107 patients were eligible for evaluation, 58 women and 49 men. Median age was 57 years (19-88). Included patients were divided into two groups; patients with continuous PPI treatment (n=56) and patients with no PPI consumption (n=51). No difference between the groups was shown regarding age or gender distribution.

The most common indication for gastroscopy was nausea and/or reflux (50%), followed by abdominal pain (26%), anemia/bleeding (11%) and miscellaneous (12%), with no differences between the groups. Outcome after examination was normal in about half of the cases (52% overall) with no difference between the groups. Esophagitis seemed to be more common among patients not treated with PPI, 23% vs 5% for patients with PPI (Fig. 12).

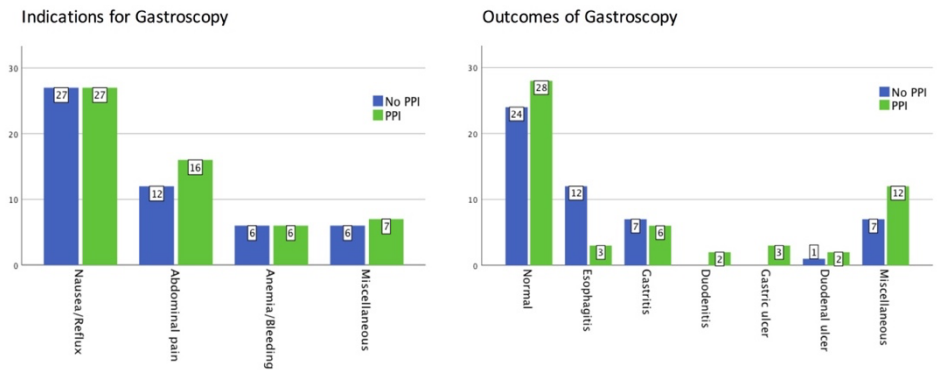

Figure 12. Indications and Outcome of gastroscopy.

3.2.2 BACTERIAL COLONIZATION

The grade of bacterial colonization and distribution between the groups show similar patterns in the stomach and in the duodenum.

Stomach: Out of the 107 patients, 57 (53%) had no bacterial colonization, 33 (31%) had slight bacterial growth and 17 (16%) had abundant bacterial growth. In the group of patients not treated with PPI (n=51), 40 (78%) had no bacterial growth, 8 (16%) had slight growth and 3 (6%) had abundant growth. In the group of patients treated with PPI (n=56), 17 (30%) had no bacterial growth, 25 (45%) had slight bacterial growth and 14 (25%) had abundant bacterial growth.

The distribution of grade of bacterial growth differed highly significantly between the two groups (no PPI/ ongoing PPI), p<0,0001. There was a significant difference between the two groups for each grade of growth.

Duodenum: Out of the 107 patients 53 (50%) had no bacterial colonization, 41 (38%) had slight bacterial growth and 13 (12%) had abundant bacterial growth. In the group of patients not treated with PPI (n=51), 39 (76%) had no bacterial growth, 8 (16%) had slight growth and 4 (8%) had abundant growth. In the group of patients treated with PPI (n=56), 14 (25%) had no bacterial growth, 33 (59%) had slight bacterial growth and 9 (16%) had abundant bacterial growth. The distribution of grade of bacterial growth differed highly significantly between the groups (no PPI/ ongoing PPI), p<0,0001. There was a significant difference between the groups in number of patients with no growth and slight growth. The number of patients with abundant growth did not differ significantly between the groups (Fig. 13).

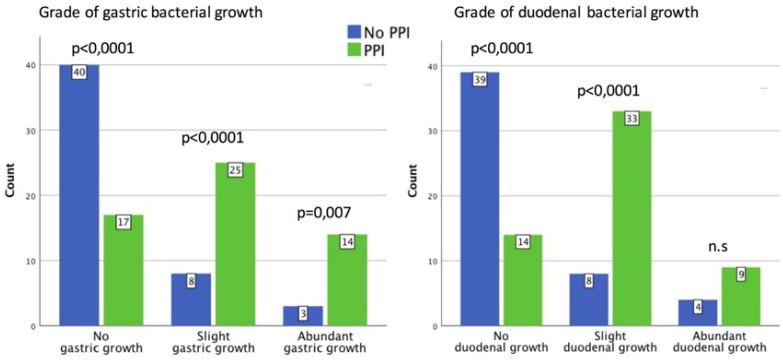

Figure 13. Grade of gastric/duodenal bacterial growth.

Stomach & Duodenum: In total 18 unique individuals showed abundant bacterial growth, 12 in both the stomach and the duodenum, 5 only in the stomach and 1 patient only in the duodenum. There was a tendency towards higher age among patients with abundant growth in the stomach (p=0,07). In the duodenum, patients with abundant growth were significantly older than patients with no growth or patients with slight growth (p=0,02).

Bacterial species: The dominating flora in the abundant bacterial colonies, both in the stomach and the duodenum, was of oropharyngeal origin, as previously described [75]. The most common species for abundant growth were of *Streptoccus mitis* and *salivarius groups,* in both locations. Most of the individuals with abundant growth had several different bacterial isolates in their samples. One individual showed growth of 7 different isolates, three had 6, one had 5, 3 had 4 isolates and so on (Fig. 14).

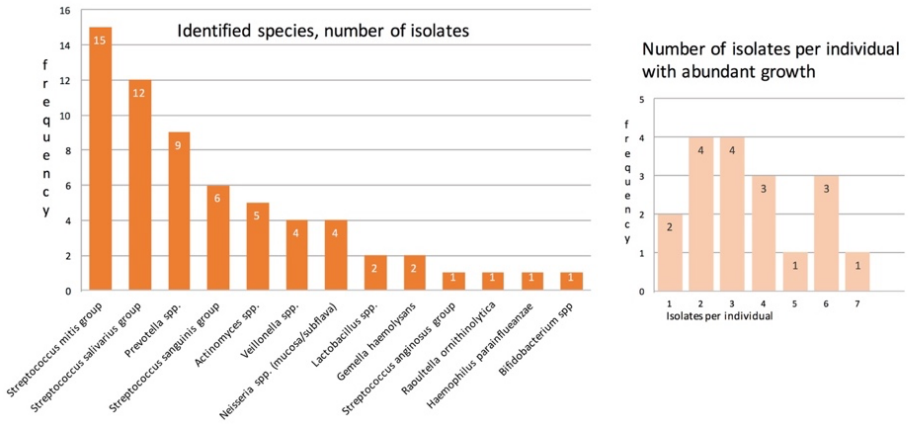

Figure 14. Identified species and number of isolates.

3.2.3 GASTRIC PH

Gastric pH was measured in 105/107 patients and varied between 1,1 and 8,3. Patients not treated with PPI (n=51) had a median gastric pH of 1,7 (1,1-8,0), while patients with continuous PPI treatment had a significantly higher pH-level of 6,9 (1,7-8,3) (p<0,0001) (Fig. 15). The distribution of gastric pH showed different patterns for the two groups. Most of the patients not treated with PPI, 90% (46/51), had a gastric pH below 4 and the remaining 5 patients had pH levels of 6,5 and above. Patients on continuous PPI treatment showed

a more varying distribution, 14/56 individuals (26%) had a pH below 4, 10/56 (18%) between 4 and 6, and 30/56 (54%) had a gastric pH of more than 6.

Age did not affect gastric pH levels in patients not treated with PPI.

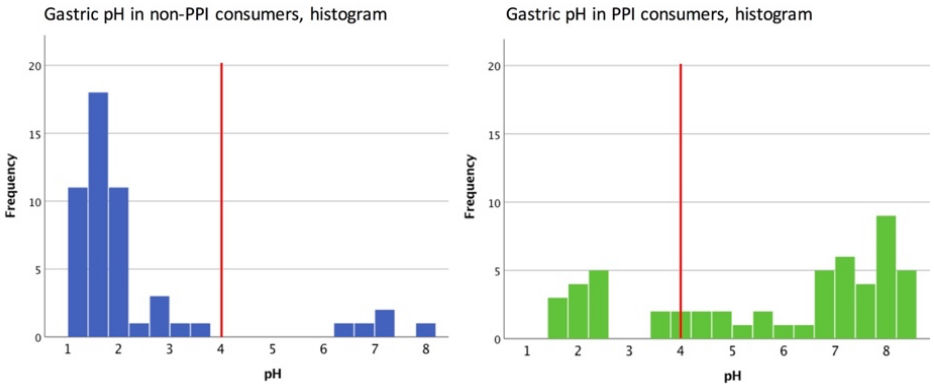

Figure 15. Gastric pH.

Gastric pH-levels differed significantly between patients with different grades of bacterial growth, both in the stomach and in the duodenum ($p<0,0001$ for both). Patients with no bacterial growth (both locations) had a median gastric pH of 1,7 with a narrow interquartile range of 1,4-2,3. For patients with slight gastric bacterial growth median pH was 6,5 with a larger variation, interquartile range: 2,7-7,5. while patients with abundant gastric bacterial growth had a median gastric pH of 7,7 with a narrow interquartile range: 6,8-8,1. Similar results were found concerning duodenal bacterial growth and gastric pH (Fig. 16).

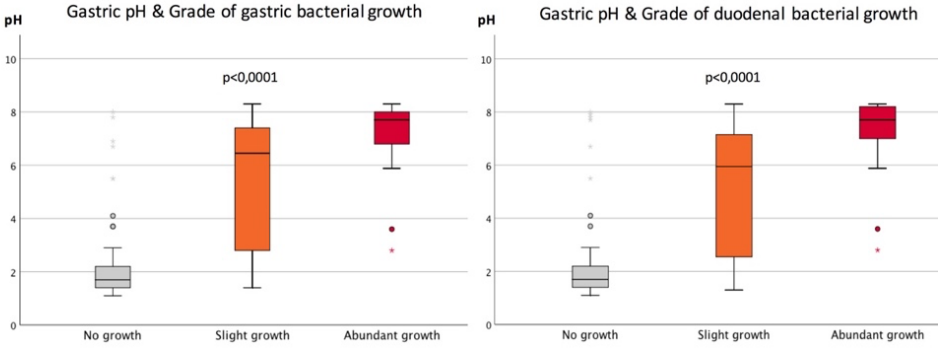

Figure 16. Gastric pH and Grade of bacterial growth.

3.2.4 PPI – SUBSTANCES AND DOSES AND THEIR EFFECT ON GASTRIC pH

Omeprazole was the most commonly used PPI in this study, consumed by 43/56 patients (77%), followed by Esomeprazole in 9/56 (16%), Pantoprazole in 3/56 and Lanzoprazole in one patient. Omeprazole was consumed as a daily dose of 20 mg in 32/56 patients (57%) and 40 mg in 9/56, in two patients the dose was not specified. Esomeprazole doses were 20 mg daily in 4/56 patients and 40 mg daily in 5/56, Pantoprazole doses were 20 mg in one patient and 40 mg in two. The single patient with Lanzoprazole used 15 mg daily. For comparisons, doses of 20 mg and 40 mg were pooled separately irrespective of PPI-substance, the single patient with 15 mg Lanzoprazole was added to the 20-mg pool. Comparing gastric pH levels for different doses of PPI, non-PPI with a 20 mg daily dose and a 40 mg daily dose, we found an overall significantly lower pH level in non-PPI consumers than in PPI-consumers ($p<0,0001$), as described above. Individuals taking 40 mg daily had a slightly higher gastric pH level than those taking 20 mg ($p=0,056$), and interestingly, with a smaller variation in interquartile range (Fig. 17).

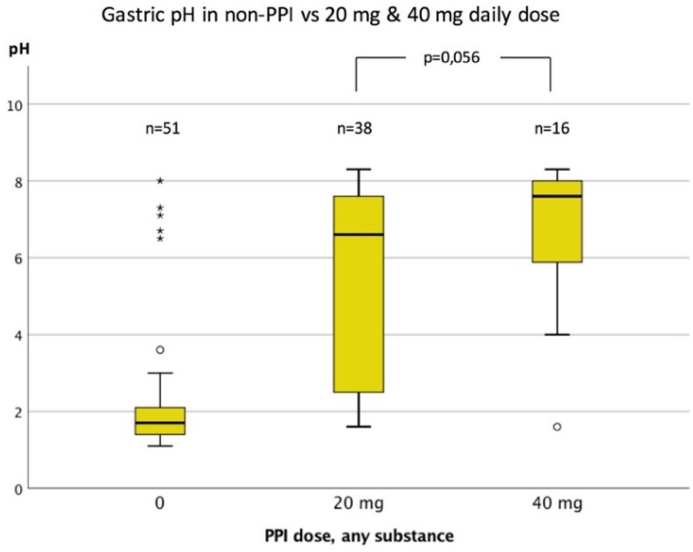

Figure 17. Gastric pH and PPI dose.

3.2.5 CONTROL-SAMPLING FROM GASTROSCOPES

Tested endoscopes used in the study showed no bacterial growth.

3.3 PAPER III

3.3.1 BASELINE PHYSIOLOGY STUDIES

With increasing probe-balloon volumes, both the pyloric pressure and minimum cross-sectional area increased, indicating a trend of increased distensibility – as expected. In all 5 pigs, cyclic variations in pyloric distensibility were recorded, seemingly more pronounced with increasing balloon volumes. Low amplitude motility waves were seen at 20 ml balloon distention. Following further inflation (30 and 40 ml) the pylorus opened wider with increasing amplitude motility waves of the same frequency. At maximum inflation (50 ml), mean pyloric pressure increased significantly (p=0,016) and the motility waves appeared flickering with hardly any amplitude (Fig. 18).

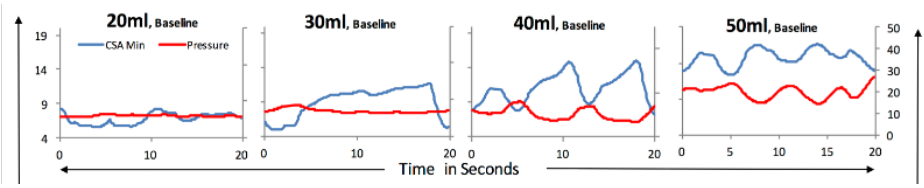

Figure 18. Pyloric diameter and pressure at baseline.

Left axis: pyloric diameter in mm. Right axis: pyloric pressure in mmHg.

3.3.2 STENT TEST

Distention of the balloon placed within a stent positioned over the pylorus, showed that low filling volumes of 20 or 30 ml did not change pyloric diameter. However, with balloon inflation of 40 or 50 ml the pylorus opens further. This pattern of increasing pressure and slight opening indicates that the pylorus becomes less distensible with the placement of a metal stent.

3.3.3 PROKINETIC TEST

Mean pyloric pressure decreased directly after administration of neostigmine. This effect was seen at all balloon volumes together with an unaltered pyloric opening diameter and for balloon-volumes 20-30-40 ml also increasing frequency in motility waves. At 50 ml balloon-volume the motility waves changed to a low amplitude flickering pattern (Fig. 19).

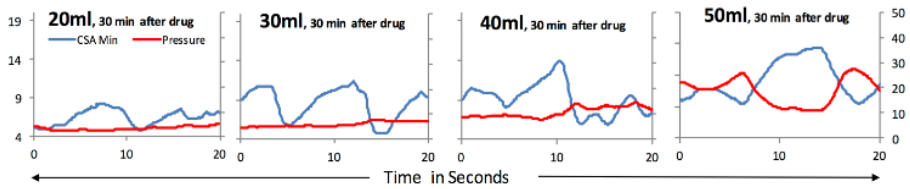

Figure 19. Pyloric pressure after neostigmine.

Left axis: pyloric diameter in mm. Right axis: pyloric pressure in mmHg.

3.3.4 LIQUID MEAL TEST

Five minutes after instillation of liquid food into the antrum the pylorus opening seemed to widen whilst the pyloric pressure stayed as low as baseline measurements. At the same time, strong motility waves passed through the pylorus with balloon volumes of 30-40 ml. However, with 50 ml balloon volume the motility disappeared leaving a flickering motility pattern with low-amplitude waves. Thirty minutes later, at balloon volumes of 20-30-40 ml, pyloric pressure kept its low level while the pyloric opening diameter decreased (Fig. 20).

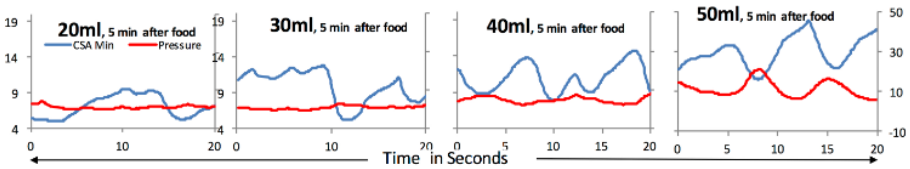

Figure 20. Pyloric pressure 5 minutes after meal.

Left axis: pyloric diameter in mm. Right axis: pyloric pressure in mmHg.

3.3.5 HUMAN VOLUNTEER

Placement of the EndoFLIP™ balloon over the pylorus was successfully performed and baseline measurements showed no changes in pyloric pressure or opening at balloon volumes of 20-30 and 40 ml. After administration of metoclopramide motility waves were recorded.

3.4 PAPER IV

3.4.1 INCLUSION AND RANDOMIZATION

Two hospitals, out of the five intended, managed to include totally 43 patients. Two patients were excluded prior to laparoscopy, one due to acute deterioration and one patient changed his mind and denied participation. Laparoscopy was performed in 41 patients. A perforated duodenal ulcer was found in 28 patients. Ten patients had other gastrointestinal perforations and in one patient no perforation was detected. Two patients were excluded due to protocol violation. Randomization resulted in 15 patients for surgical treatment and 13 for stent treatment (Fig. 21).

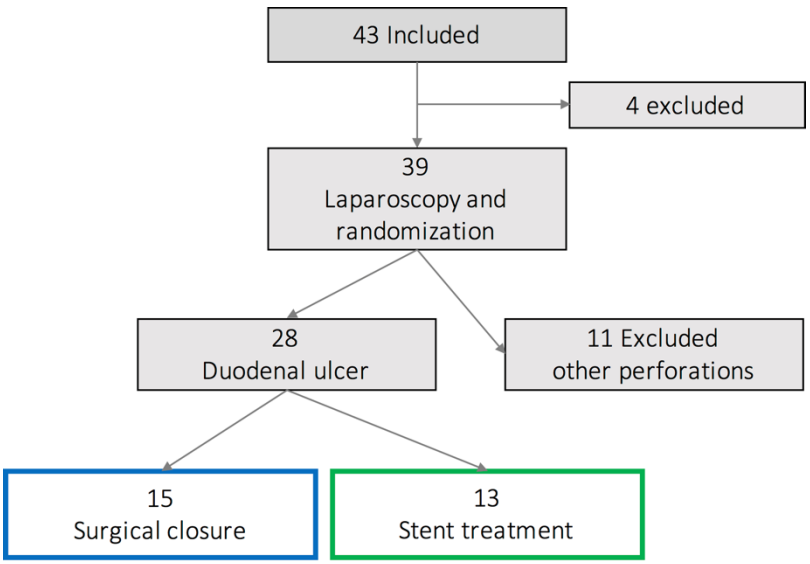

Figure 21. Inclusion and randomization.

3.4.2 DEMOGRAPHICS

There were no significant differences between the groups concerning age, gender or BMI, see table 1 for details. Interestingly, included women were slightly older than included men, 82 years (37-89) vs. 74 years (23-91) (n.s.) (Table 1).

Demographic data	*Surgical closure*	*Stent treatment*	*All patients*	
Number	15	13	28	
Age, years median (range)	75 y (23-91)	80 y (38-87)	77y (23-91)	n.s.
Gender, Female/Male	8F / 7M	7F / 6M	15 F / 13 M	n.s.
BMI, kg/m^2 median (range)	28 (21-30)	24 (19-30)	27 (19-30)	n.s.

Table 1. Demographic data for the 28 included patients.

3.4.3 ASA-SCORE

Patients in the surgical closure groups had an ASA-score of 1-3 while patients in the stent group had an ASA-score of 1-4, showing a tendency to difference but not significantly (p=0,069). All ASA 4 patients were randomized to the stent group (Fig. 22).

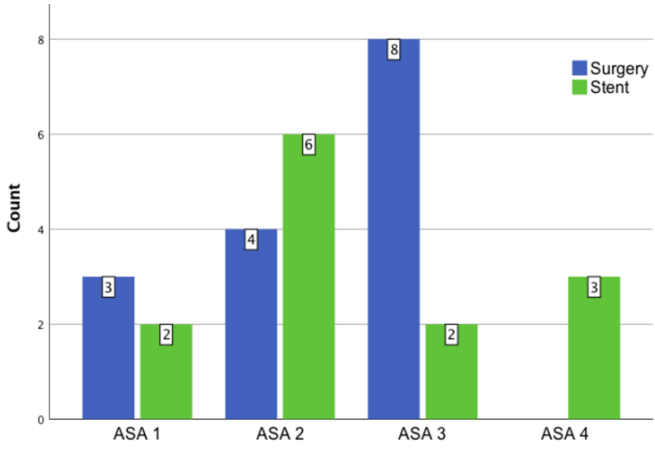

Figure 22. ASA scores presented by treatment group.

3.4.4 TIME TO INTERVENTION

Twelve patients were operated after more than 12 hours from symptom onset, 5/15 in the surgical group and 7/13 in the stent group (n.s.).

3.4.5 SURGICAL CLOSURE TECHNIQUE

Of the 15 patients randomized to the surgical group, 5 were operated using laparoscopic technique and 10 were converted to open surgery.

3.4.6 OPERATION TIME

Median operation time was 92 minutes (68-154) for surgical closure and 68 minutes (48-107) for stent treatment (p=0,001). For the stent group, operation time included both diagnostic laparoscopy and gastroscopy with stenting. For surgical closure operation time included diagnostic laparoscopy and surgical closure (Fig. 23).

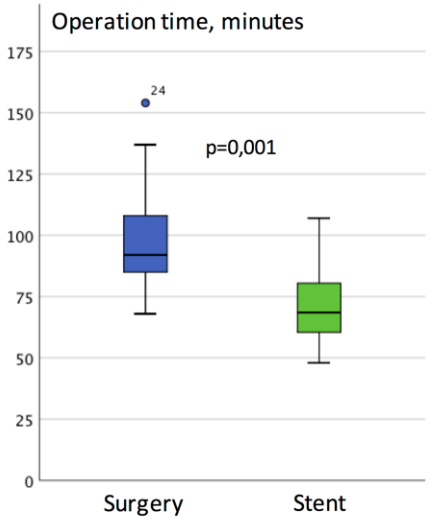

Figure 23. Operation time.

3.4.7 CRP AND WBC

There was no significant differences between the groups regarding post-operative follow-up of CRP and WBC. All patients had a significant rise in CRP on postoperative day (Fig. 24).

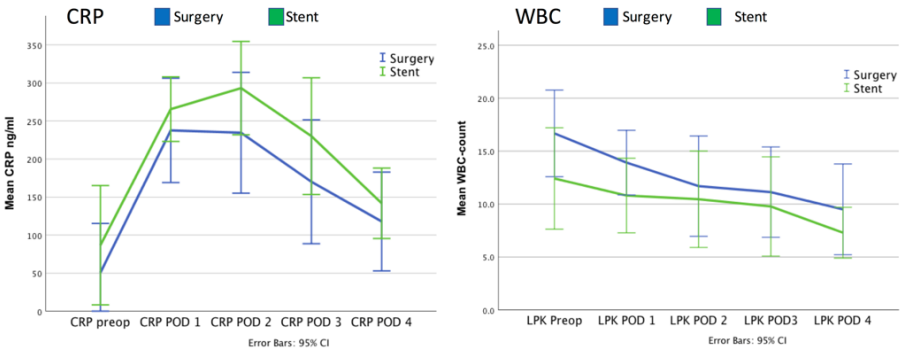

Figure 24. C-reactive protein and White blood cell count.

3.4.8 HOSPITAL STAY

Hospital stay did not differ between the groups. Median stay was 8 days (2-27) for stent treatment and 7 days (3-24) for surgery (Fig. 25).

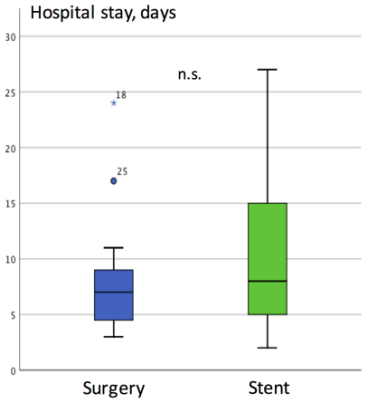

Figure 25. Hospital stay.

3.4.9 STENT REMOVAL

Stent removal was performed 21 days (11-37) after initial treatment, without adverse events.

3.4.10 MORBIDITY AND MORTALITY

Overall morbidity (complication Clavien-Dindo grade 2-4) was 12/28 (42%) and mortality was 1/28 (4%), without differences between the groups. Six patients in each group had a Clavien-Dindo 2-4-complication.

3.4.11 COMPLICATIONS

- *Surgical closure*

Two patients had post-operative fever and one had pneumonia (C-D 2). One patient had a surgical site leakage, diagnosed at the leakage test on postoperative day 1, and was treated with a covered stent. This patient also developed an abscess, at the leakage site, that was drained percutaneously (C-D 3). Two patients needed prolonged postoperative intensive care due to renal and circulatory failure (C-D 4). One of these patients developed a stricture at the surgical closure site and needed total parental nutrition.

- *Stent treatment*

Two patients had an intraabdominal abscess, both drained percutaneously (C-D 3). One patient showed leakage at leakage test and received a new stent without further complication (C-D 3). One patient with circulatory failure and two with a combination of circulatory and renal failure needed postoperative intensive care (C-D 4). One patient with preoperatively deteriorated clinical

condition and a more than 7-day long history of abdominal pain, presenting in a septic condition, developed multi organ failure and died (C-D 5) (Fig. 26).

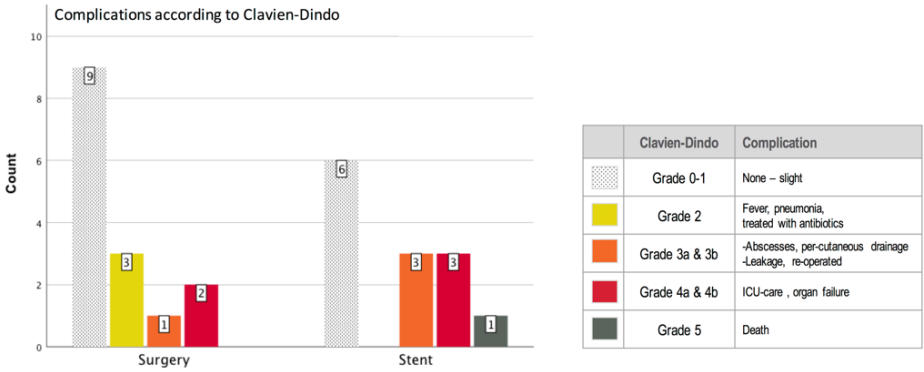

Figure 26. Distribution of complications, defined according to Clavien-Dindo.

3.4.12 COMPLICATIONS IN RELATION TO AGE

Patients with a complication Clavien-Dindo 3-4-5 (n=10) were significantly older than patients with Clavien-Dindo 0-1-2. Median age in this group was 84 years (73-91) (p=0,016).

3.4.13 COMPLICATIONS IN RELATION TO TIME TO INTERVENTION

Patients treated more than 12 hours after symptom onset had significantly more complications Clavien-Dindo grade 3-5 (p=0,04). Three out of the four patients with a Clavien-Dindo 3 complication (abscesses), and 3/5 patients with a Clavien-Dindo-4 complication were operated after more than 12 hours with symptoms. The patient who died also had a late intervention (Fig. 27).

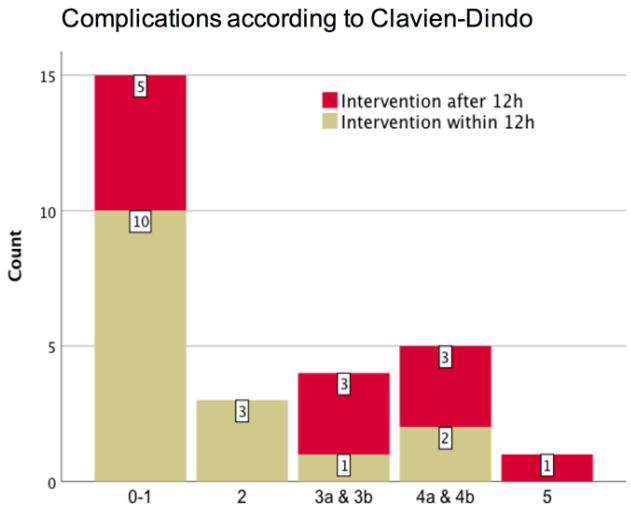

Figure 27. Complications in relation to time to intervention.

3.4.14 COMPLICATIONS AND HOSPITAL STAY

Overall, patients with delayed intervention of more than 12 hours from symptom onset had longer hospital stay than those operated within 12 hours ($P < 0.013$). Median stay was 13 days (4-27) for those with delayed intervention more than 12 hours compared with 6 days (2-11) for those treated within 12 hours.

Hospital stay was significantly longer in patients with complication (C-D 2-5) than those without ($P = 0.001$) Hospital stay was 15 days (6-27) for patients with complications compared to 5 days (2-8) for those without complications. Intervention group did not affect this difference.

4 DISCUSSION

Surgical closure is still the gold standard treatment of perforated duodenal ulcer, since Mikulicz-Radecki introduced the technique in 1885 [15]. Today this procedure is often performed by laparoscopy, a minimal invasive technique for accessing the abdominal cavity, carrying certain advantages regarding postoperative outcome [18]. However, the surgical principle of operative sutured closure remains [76].

We present an endoscopic treatment alternative using a covered self-expandable metal stent (SEMS). It was initially used in 2 patients with leakage after surgical closure, followed by a series of 6 patients who were poor surgical candidates. As these results were encouraging [51] we decided to perform a randomized study comparing surgical closure with stent treatment.

Power calculation was difficult to perform as there was no previous scientific data, besides our small case series. According to our estimations, 50 patients would be needed in each group to show non-difference. We planned to achieve full inclusion within 5 years, based on a Nordic incidence of perforated duodenal ulcer of 2/100 000 inhabitants/year [16], resulting in 30 cases/year in our region (Västra Götaland Region). To increase inclusion rate, 5 centers within our region had accepted to participate in the study. However, inclusion was demanding mostly for logistic reasons, such as lack of competent endoscopist during non-office hours, resulting in inclusion at only two out of the five intended surgical centers. Another reason for low inclusion rate might be that only patients able to sign the consent form were eligible for inclusion, excluding patients not understanding Swedish or patients in poor clinical condition such as circulatory failure or disorientation. The decreasing incidence of perforated duodenal ulcer might also have contributed to fewer cases than expected, leading to a lower inclusion rate. According to the study protocol, a safety analyses was planned halfway through inclusion. Due to the low inclusion rate, we decided to perform an intermediate analysis after four years, despite fewer cases than anticipated.

Free air shown on a CT-scan, or on a plain abdominal X-ray, indicates a visceral perforation but cannot with certainty deduct its origin. Taylor's method is based on the clinical observation that the omentum together with the liver will cover most of gastric or duodenal perforations, why surgery might not be needed in many cases [77]. Studies of conservative treatment of perforated peptic ulcers are difficult to evaluate as treatment usually is assigned and given without an objectively verified diagnosis. Crofts performed a

randomized trial during the 80s, comparing non-operative treatment for perforated peptic ulcer with surgery, including 83 patients. The diagnosis was established through clinical history and clinical signs, some with free air on X-ray, 40 patients were randomized to conservative treatment. A radiologic study with water soluble contrast was performed in 38 out of these 40 patients to confirm diagnosis, but the number of patients with contrast leakage or verified ulcer is not given in the paper [78]. In our study, 25% (11/41) of the patients showed a non-duodenal site of perforation at laparoscopy, despite clinical signs of upper G-I tract perforation and free air on a CT-scan, indicating the difficulty in preoperative diagnostics. In previous studies of conservative treatment, many different diagnoses may have been included. It is difficult to decide the origin of free air even on a CT-scan why we performed laparoscopy in all randomized patients to verify the diagnosis, adding strength to our study.

All patients in this study were evaluated according to the ASA-score. It seems that patients with higher pre-operative comorbidity by coincidence ended up in the stent group, resulting in a tendency towards higher ASA-score in the stent group $p = 0.069$ (χ^2 test). This uneven distribution might have affected morbidity and mortality for the stented patients in a negative direction. However, the numbers are too small to allow such a conclusion.

Delayed time to operation, long operation time together with high age, co-morbidity and septic shock on arrival have been shown to increase morbidity and mortality after perforated duodenal ulcer [11, 14, 19, 79]. The complication rate in this study is similar to reports in literature [11, 14] and does not differ between the two treatment groups. Instead the complication rate was related to delayed time to intervention of more than 12 hours and to patient´s age. We could show that a C-D 3-5 complication correlated statistically with delayed intervention, more than 12 h from symptom onset (p=0,004). In our study, stent treatment showed significantly shorter operation time than surgical closure (p=0,001). Several review articles emphasize the importance of shortening operation time, especially in patients with a high ASA-score or in septic shock, to minimize morbidity and mortality [19]. Therefore, stent treatment may be a good alternative in patients where surgery appears technically challenging.

Lavage of the abdominal cavity can be technically challenging and is believed to be more difficult when performed laparoscopically. Lau proposed that intraabdominal collections might be more prevalent after laparoscopic ulcer repair, nevertheless no significant difference was shown in Lau's study [18]. In our study 3 patients (10,7%) had a postoperative abscess, 2 of them in the stent group, and one in the surgical group initially operated with laparotomy.

Literature reports show that the incidence of abscess formation after surgery for perforated peptic ulcer varies between 0-9% according to Bertleff [15], but has been described as high as 13% [80]. Post-operative abdominal abscesses in patients treated conservatively for perforated peptic ulcer occurs in about 14% according to Marshall [81].

Abscess formation in the abdominal cavity was a minor clinical problem in our study, as these patients were successfully treated with a percutaneously placed drainage. The abdominal drainage placed during laparoscopy in the current study, was not intended to prevent abscess formation but to reveal signs of leakage. Whether stent treatment of perforated duodenal ulcers can be utilized without laparoscopically performed lavage is still an unanswered question. Perhaps laparoscopy can be avoided in selected patients with short time from symptom onset to intervention and with minor amounts of free abdominal fluid, but further investigations are needed.

Leakage at the site of perforation is described in 3-6% after surgical closure [19, 82, 83]. In our study two patients had a leakage, one in each group. The patient from the surgical closure group was treated with placement of a stent, covering the site of perforation. The primarily stented patient had a replacement stent with better localization. Both patients showed good recovery without further leakage. Considering these two patients and two of the patients from our previous case series [51] stent treatment might be a safe alternative to reoperation in case of leakage from the perforation site. Some authors have treated postoperative leakage conservatively with nasogastric tube, according to Taylor's method, causing several weeks of fasting but with eventual good results [84] With a covered stent, the patients can start oral intake of liquids soon after stent placement, resulting in quicker recovery and shorter hospital stay.

Stents placed in the G-I tract are typically placed over a stricture decreasing the risk of migration. Covered stents are more prone to migration than uncovered. When stenting is performed to treat a perforation, a covered stent is necessary and is placed without the retention of a stricture, both factors increasing the risk of migration. In our previous case series of patients treated with duodenal stent we observed one case of stent migration. In that case, without fatal consequences as the stent had migrated upwards into the stomach and easily was withdrawn at gastroscopy [51]. There were no complications related to stent migration in our randomized study (paper IV).

The company MI-tech (Seoul, Korea) designed a modified version of their partially covered duodenal stent featuring a larger and wider proximal flare, the "big cup stent" (Hanaro DPC stent). The cup, placed prepylorically in the antrum, aimed at preventing stent migration and increasing tissue ingrowth. Van der Berg started a study on this stent in malignant gastric outlet obstruction in 2012 [61]. The study was prematurely terminated after 5 months due to high migration rate. Half of the patients showed stent migration into the stomach, which was tentatively explained by the "soap-bar effect" [85], secondary to retrograde peristalsis and a conical stent shape, tossing the stent into the stomach. Postprandial retrograde duodenal motility waves were described by Castedal et al [62]. We hypothesize that the large flares of the "big-cup-stent" provoked pyloric motility by local stimulation of the prepyloric antral area. In our study, we used a traditional duodenal stent with a shallow and short flares, less provoking to the prepyloric area, partially explaining the lack of migration. There are also studies showing a decrease in negative duodenal motility in the presence of a duodenal ulcer [86], an effect that might have contributed to the lack of stent migration in our randomized study.

In order to investigate how a stent may affect pyloric motility we performed a study using the EndoFLIP™ (paper III). By step-wise inflating the FLIP-balloon, exerting increasing pressure and dilatation of the pylorus, we mimicked the effect of stents with different radial forces. We could show that increasing dilatation of the pylorus induced increasing motility waves and the pylorus started acting like a peristaltic pump instead of acting as a sphincter, just opening and closing an orifice. When the pylorus was fully distended the motility almost disappeared, leaving the pylorus wide open with low amplitude flickering motility waves. These results indicate that a duodenal stent placed over the pylorus should have a high-radial force in order to dilate the pylorus and reduce the contraction waves, thus reducing the risk of migration.

We also studied how food affects pyloric motility with the EndoFLIP™ balloon in place, mimicking a stent. With liquid food placed in the antrum, high-amplitude contraction waves were recorded together with increasing pyloric diameter showing increased motility and relaxation of the pylorus. Our interpretation of this motility pattern is that the antrum and pylorus contributes to stomach emptying when stimulated. It has been shown that solid food increases antral motility, working and mixing the gastric content until it can be swept through the pylorus with a motility wave. Liquids pass the pylorus with the motility wave while solids stay in the stomach for further processing [87]. Intake of solid food, even well chewed, may also obstruct the stent lumen, increasing the risk of migration. Considering this, we kept our patients on liquid food to reduce the risk of stent migration. Intake of solid food, even well

chewed, may also obstruct the stent lumen, thus increasing the risk of migration. The regime of liquid food during stent treatment was well tolerated by the patients.

Peptic perforations are believed to resemble clean-contaminated cases, due to the expected low pH in the stomach creating a sterile environment [88]. Since the introduction of proton pump inhibitors during the 90s, consumption has increased manifold and today the extensive use creates a large population of patients with high intra gastric pH. A gastric pH of more than 4 has been shown to allow bacterial colonization [68]. In our studies of bacterial colonization of the stomach and duodenum (papers I &II) we found that patients on continuous PPI medication significantly more often showed bacterial growth, both in the stomach and in the duodenum. This finding was expected, as PPIs increase gastric pH.

In both studies, oropharyngeal bacterial flora dominated in both gastric and duodenal samples. Dominating species were: *Streptococcus salivarius* and *mitis*, followed by *Neisseria* species *(mucosa/subflava*) and *Streptococcus parasanguinis*. Our hypothesis is that bacteria are swallowed along with food or saliva. Interestingly PPI users have been shown to have increased amounts of oropharyngeal flora in samples from stools [89], affecting the gut microbiota, a finding supporting our hypothesis. Bacteria of oro-pharyngeal origin are not locally pathogenic in the stomach, but may contaminate the abdominal cavity if a perforation occurs or during transgastric interventions [72, 73]. In a retrospective analysis of all patients treated for perforated duodenal ulcer at our hospital during 2009-2018, abdominal cultures were obtained at surgery from 24/98 patients. Twelve of these 24 patients had a positive abdominal culture and the flora was of oropharyngeal origin, dominated by *streptococcus salivarius* and *mitis* together with *Candida species* (unpublished data).

No acid, no ulcer is an old axiom [4]. This is still true, but today we know that *Helicobacter Pylori* and the widespread consumption of NSAIDs also contribute to ulcer disease due to impaired gastric mucosal cytoprotection. In our study, in paper I, 10 % had a positive urease test indicating presence of *Helicobacter Pylori*. This is in line with previously shown incidence of *H. pylori* in the Nordic population [90].

Measurements of gastric pH was performed in the second study on bacterial colonization (paper II), to confirm its correlation both with PPI-intake and with bacterial growth. We found a clear correlation between pH > 4 and bacterial

colonization. As expected PPI-consumers had significantly higher gastric pH than non-PPI consumers ($p<0,0001$).

Interestingly patients with abundant gastric and/or duodenal bacterial growth had high pH levels within a small inter-quartile range. Eighteen individuals showed abundant gastric and/or duodenal bacterial growth. Most of them (15/18) were PPI consumers and had a gastric pH of 6 and above. The remaining three individuals were non-PPI consumers, one 36-year-old who had a gastric pH of 7, and finding of gastritis at endoscopy. This is in line with previous findings where atrophic gastritis is associated with gastric bacterial overgrowth [91]. Two non-PPI consuming individuals with abundant bacterial growth were relatively old, 78 & 80 years, and had gastric pH levels below 4 (pH 2,8 & pH 3,6). Other factors damaging the gastric mucosal barrier without affecting gastric pH, such as *H. pylori* [92, 93] or consumption of NSAIDs [94], may have been present facilitating local bacterial colonization. Both these patients had an endoscopic diagnosis of gastritis together with supportive findings at histology.

Historically, atrophic gastritis was believed to increase with age, with a concomitant rise in gastric pH. Recent studies have contradicted these theories [95, 96]. In line with this, our study shows no age dependent rise in gastric pH in non-PPI consuming individuals. However, there seems to be an increasing propensity for bacterial colonization with age, independent of gastric pH. This tendency was observed in both paper I and II. Bacterial overgrowth in the gastrointestinal tract has been associated with gastric achlorhydria and motility disorders with poor gastric emptying in the elderly [97]. It seems reasonable that slow gastric emptying and slow further transit of gastric content facilitates gastric and duodenal bacterial colonization from swallowed saliva.

Gastric pH varied within a large range in patients with PPI treatment (Fig. 15). The acid reducing effect of PPIs is dose dependent. The pH increasing effect has been shown to last for 12-14 hours with individual variations [98]. There are also data suggesting ethnical differences in PPI metabolism and effect [99]. In our study, 66% of PPI-consumers utilized a 20-mg daily dose of Omeprazole or an equivalent dose of another PPI [99, 100]. Many patients take their PPIs in the morning but omit their medication on the day of gastroscopy, allowing for a decrease in gastric pH before the examination. Variations in individual response, doses and time of daily administration may explain the large variation in gastric pH-levels for patients treated with PPI in this study.

Less variations in gastric pH levels were seen in patients without PPI consumption, who showed a consistent pattern in gastric pH levels. A large

majority (90%) had a gastric pH level below 4, with 42/56 below pH 2. The remaining 5 individuals (9%), showed pH levels above 6,5 possibly indicating atrophic gastritis, without correlation to age.

In the case of a peptic perforation, contamination of the abdominal cavity may occur, as shown in different studies [72, 73]. It is important to begin adequate and efficient antibiotic treatment, bearing present findings in mind. The choice of antibiotics ought to focus on bacteria usually found in the oral cavity like streptococci, especially in patients with ongoing PPI treatment. An antibiotic with narrow spectrum might be sufficient, instead of the often-used broad spectrum antibiotics.

Mortality rates for peptic ulcer perforations seem to be stable despite current decline in ulcer disease incidence. Predisposing factors are still prevalent, such as *H. pylori*, smoking, NSAIDs, alcohol, drug abuse and fasting. For more than two centuries, sutured surgical closure has been the gold standard treatment. We present an endoscopic treatment option, using a covered metal stent, constituting a minimal invasive alternative. Regardless of therapeutic technic, short time to intervention seems to be crucial for a good outcome.

5 CONCLUSION

Bacterial flora found in the stomach and/or duodenum is mainly of oropharyngeal origin, more frequently occurring in patients with ongoing PPI treatment. Similar bacteria were found in the abdominal cavity after ulcer perforations.

Individuals with high gastric pH, often due to PPI intake, are more at risk for abundant gastric and/or duodenal bacterial colonization.

Stent design influences pyloric motility, through pyloric distention, and seems to be of importance to avoid stent related complications.

Bearing the small sample size in mind, stent treatment of perforated duodenal ulcer seems to be as safe and effective as surgical closure regarding postoperative morbidity and mortality.

Stent treatment might be advantageous in patients presenting postoperative leakage after sutured closure, avoiding repeat surgery. Stent treatment is also a valuable alternative in patients where surgical closure is difficult due to location, previous surgery or patient habitus.

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