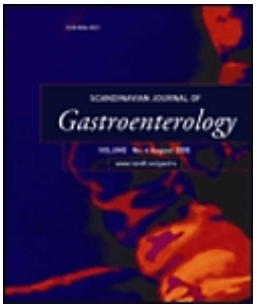

Narrow band imaging and serology in the assessment of premalignant gastric pathology

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Title Page

Narrow band imaging and serology in the assessment of premalignant gastric pathology

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Keywords

Endoscopy, narrow band imaging, white light endoscopy, serology, *H. pylori* gastritis, gastric atrophy, intestinal metaplasia

Abstract

Background

Patient outcomes in gastric adenocarcinoma are poor due to late diagnosis. Detecting and treating at the premalignant stage has the potential to improve this. *H. pylori* is also a strong risk factor for this disease.

Aims

Primary aims were to assess the diagnostic accuracy of magnified narrow band imaging (NBI-Z) endoscopy and serology in detecting normal mucosa, *H. pylori* gastritis and gastric atrophy. Secondary aims were to compare the diagnostic accuracies of two classification systems using both NBI-Z and white light endoscopy with magnification (WLE-Z) and evaluate the inter-observer agreement.

Methods

Patients were prospectively recruited. Images of gastric mucosa were stored with histology and serum for IgG *H. pylori* and Pepsinogen (PG) I/II ELISAs. Blinded expert endoscopists agreed on mucosal pattern. Mucosal images and serological markers were compared with histology**.** Kappa statistics determined inter-observer variability for randomly allocated images among four experts and four non-experts.

Results

116 patients were prospectively recruited. Diagnostic accuracy of NBI-Z for determining normal gastric mucosa was 0.87(95%CI 0.82-0.92), *H. pylori* gastritis 0.65(95%CI 0.55-0.75) and gastric atrophy 0.88(95%CI 0.81-0.94). NBI-Z was superior to serology at detecting gastric atrophy: NBI-Z gastric atrophy 0.88(95%CI 0.81-0.94) vs PGI/II ratio<3 0.74(95%CI 0.62-0.85) p<0.0001. Overall NBI-Z was superior to WLE-Z in detecting disease using two validated classifications. Inter-observer agreement was 0.63(95%CI 0.51-0.73).

Conclusions

NBI-Z accurately detects changes in the GI mucosa which currently depend on histology. NBI-Z is useful in the detection of precancerous lesions, potentially improving patient outcomes with early intervention to prevent gastric cancer.

Introduction

Helicobacter pylori (*H. pylori*) colonizes the gastric mucosa of approximately 50% of the world's population, although the prevalence varies between countries the infection rates are higher in developing countries (1, 2). *H. pylori* infects individuals during childhood and typically persists lifelong in the absence of effective eradication therapy (3). In 15% of individuals infection leads to serious complications such as peptic ulcer disease, distal gastric adenocarcinoma or primary gastric mucosa associated lymphoid tissue (MALT) lymphoma (4). Other conditions associated with *H. pylori* include iron deficiency anemia, gastric atrophy and idiopathic thrombocytopenia purpura (5, 6). *H. pylori* infection has also been suggested to be a significant contributor to the hygiene hypothesis and the development of a healthy immune system (4, 7).

Gastric atrophy occurs as a result of a chronic gastric mucosal inflammation, usually due to *H. pylori* infection, leading to loss of specialized glandular cells and replacement with an intestinal type surface and fibrous tissue (8). This may then progress to intestinal metaplasia then dysplasia and finally intestinal type gastric cancer. The development from normal mucosa to gastric cancer is gradual and in a stepwise manner (9). The annual incidence of progression from intestinal metaplasia to gastric cancer varies from 0.1% to 0.9% (10). The annual incidence increases to 6% in dysplasia (11, 12). Gastric atrophy is, therefore, considered a pre-cancerous condition and current European guidelines recommend endoscopic surveillance of these high risk patients with

severe atrophy (13). Unfortunately, at present these premalignant mucosal changes are often disregarded in routine clinical practice or result in variable surveillance frequency (14). Gastric cancer is ranked the fifth most common malignancy worldwide (15) and is the third most common cause of cancer related death (16). In order to reduce this associated mortality, one possibility is to detect premalignant disease using advanced endoscopy techniques. However, due to variety in endoscopic techniques and mucosal classifications the diagnosis is often dependent on histology.

High definition magnified white light endoscopy (WLE-Z) allows for detailed assessment of mucosal pit and vascular pattern, magnifying images by factors greater than 100 with resolutions smaller than 7.9µm (17-19). Narrow band imaging (NBI) relies on specific wavelengths of light to produce a sharper contrast between mucosal and vascular structures enhancing detail (20, 21). NBI endoscopes enable the endoscopist to easily switch from WLE to NBI to optimize visualization and sampling.

Standard WLE mucosal appearances often correlate poorly with histology and there is no widely accepted consensus on the macroscopic appearance, therefore, clinicians are reliant on histology which is dependent on multiple factors such as biopsy area, biopsy numbers and experience of the histopathologist (22, 23). Anagnostopoulos *et al* proposed a classification using WLE-Z which was highly accurate for detecting normal, *H. pylori* gastritis and gastric atrophy with sensitivity and specificity of 90-100%. Using this classification a normal gastric corpus microvasculature consists of a honeycomb type sub epithelial capillary network (SECN) with a regular arrangement of collecting venules and round pits. In the inflamed corpus, the normal SECN pattern and collecting venules are lost with enlarged white pits surrounded by erythema. Gastric atrophy is characterized by loss of the normal SECN and round pits, with an irregular distribution of collecting venules (18). Pimentel *et al* described a more detailed NBI classification that also included intestinal metaplasia and dysplasia with accuracy of 84% and 95% respectively (24). The light blue crest (blue-whitish patchy reflections sited on the epithelial margins) appearance in magnified NBI (NBI-Z) of gastric mucosa was first described by Uedo *et al* and correlated well to intestinal metaplasia with an accuracy of 91% (25). White Opaque Substance (WOS) is also sometimes associated with epithelial tumors and intestinal metaplasia

(26). The use of NBI in the detection of these conditions was also accurate in other studies but due to study limitations reported results vary (27-30).

IgG ELISA serology detects *H. pylori* antibodies and is relatively cheap and non-invasive but a positive test cannot distinguish between current and previously treated infection. Accuracy is variable but some commercial kits report accuracy of greater than 90% (31) with the benefit that results are not affected by proton pump inhibitor (PPI) treatment.

Serum pepsinogen can be used to predict the extent of gastric atrophy. Pepsinogen I (PGI) and Pepsinogen II (PGII) are usually released from secretory cells found in the gastric mucosa. With the progression to atrophy this causes the loss of these secreting cells and reduces pepsinogen levels. PGI is more affected by this and thus the ratio is decreased further. Low pepsinogen I or pepsinogen I/II ratio less than 3 can signify moderate to severe corpus atrophy (13). For estimating gastric atrophy extent studies have described a huge variation in results with sensitivity and specificity from 9.4% to 92.3% and 9.9% to 100% respectively (13, 32-34). Serological markers of gastric atrophy are not routinely used in Western countries.

No study has yet evaluated the utility of both magnified narrow band imaging and serology in the detection of premalignant gastric pathology. Patients referred for endoscopic investigation for iron deficiency anemia were selected for this study due to the association with both *H. pylori* and gastric atrophy (6, 35, 36). The Anagnostopoulos *et al* classification was adapted to use NBI-Z rather than WLE-Z in this study and termed the Nottingham classification.

The primary aim of this pilot study was to assess the diagnostic accuracy of magnified NBI endoscopy and serology in diagnosing normal mucosa, *H. pylori* gastritis and gastric atrophy using the Nottingham classification. Secondary aims were to compare the diagnostic accuracies of two validated classification systems using both NBI-Z and WLE-Z and evaluate the inter-observer agreement.

Material and Methods

Participants and clinical samples

150 adult patients (18-85yrs) attending Nottingham University Hospitals (NUH) NHS Trust for a diagnostic gastroscopy as part of their investigation into iron deficiency anemia were

prospectively recruited between August 2010 and December 2014. Derbyshire Research Ethical Committee (REC Ref: 10/H0401/33) approved the protocol and written informed consent was gained. Patients with low hemoglobin (<130g/l men & <120g/l women) and either a low MCV (<84fl) or low ferritin (<25mcg/l men & <13 mcg/l women) were recruited. Patients taking anticoagulants and proton pump inhibitors or individuals for whom biopsy sampling was contraindicated were not recruited. Patients who had an overt cause for iron deficiency anemia after both upper and lower GI investigations (e.g. malignancy (n=22), coeliac disease (n=9), poor quality digital images (n=2) or patients without biopsies (n=1)) were excluded. 116 patients were included in the final analysis.

Laboratory Investigations

Fasting blood samples were collected and analyzed for full blood count, B12, folate, serum ferritin, transferrin saturation, transferrin, serum iron and iron binding capacity. Plasma was also stored at -80°C until processed for *H. pylori* IgG (Biohit), PG I (Biohit) and PG II (Biohit) with ELISA kits according to manufacturer's instructions.

Endoscopy procedures

Procedures were performed by expert endoscopists using pharyngeal local anaesthetic spray Xylocaine (AstraZeneca, Luton, UK) or conscious sedation (midazolam/pethidine) according to patient preference. A black soft rubber hood (MB46, MAJ-1990, Olympus) was attached to the endoscope tip to allow a fixed 2mm distance between gastric mucosa and gastroscope. All procedures were done with high definition and magnification Gastroscopes (GIF-FQ260Z; Olympus Optical, Tokyo, Japan) and Lucera Elite CV290 video processor. The video images were viewed on a high definition video monitor (OEV-191H, Olympus). During the procedure the mucosa was washed with a mixture containing 100ml of water mixed with 2ml of acetylcysteine (200mg/ml, Parvolex, Celltech, UK) and 0.5ml (40mg/ml) dimethicone (Infacol, Forrest Laboratories, UK). Detailed examination of the gastric mucosa was then carried out, in WLE and then NBI using both low magnification and magnified views. Still digital images were recorded in both WLE and NBI, with biopsies taken from the areas where the digital images were recorded. A minimum of 8 images were recorded for each patient. All still images were transferred to an external hard drive.

Post endoscopy image production

Images were stored as JPEG files (200-300 kilobytes, 1093x948 pixels, 32-bit color), edited, anonymized and given random numbers generated in Excel Office 2010 (Microsoft Corporation, Redmond, Washington, USA) before transfer into an evaluation set of folders according to area and classification system. Two principal endoscopists (Ragunath/Sami), experts in advanced endoscopy, agreed on the magnified appearance of each selected images of the gastric mucosa in WLE-Z and NBI-Z according to specific criteria. Both were blinded to clinical, histological and serological findings. Images were reviewed and graded according to mucosal morphology using the classifications described below. These scores were used to assess magnification endoscopy performance in terms of sensitivity, specificity, accuracy, positive and negative likelihood ratios.

Image classification

Two validated classification systems were used for the gastric corpus: the Nottingham classification (Figure 1) (18) and a modified Pimentel-Nunes *et al* classification (24) to include gastric atrophy (Db) termed the modified Porto classification (Table 1, Figure 2).

Inter-observer variability study

Eight blinded endoscopists assessed the digital still images: four experienced, fully accredited endoscopists (experts) and four trainee endoscopists (non-experts). Prior to grading the images all endoscopists underwent a comprehensive, self-directed training package which covered all the gastric mucosa classifications. Each endoscopist viewed folders containing WLE-Z and NBI-Z images of the corpus for both classifications. Approximately 464 anonymized randomized images were reviewed over an unrestricted length of time. Data on image classification from a drop down menu of pre-defined options and image quality according to a 10 point Visual Analogue Scale (VAS) (37) were recorded onto an Excel spreadsheet.

Histopathology analysis

Two gastric antrum, two gastric corpus and four duodenal samples were fixed in formalin, then embedded in paraffin and cut into approximately 4μm sections. Sections were stained with

haematoxalin and eosin (H&E) to allow histological scores to be carried out according to the updated Sydney scoring system (38). All specimens were classified as none (0), mild (1), moderate (2) or severe (3) for the following histological features activity (polymorphonuclear cell infiltration), inflammation (mononuclear cell infiltration), atrophy (loss of specialized glands) and intestinal metaplasia (replacement gastric mucosa by metaplastic columnar absorptive cells and goblet cells with intestinal morphologic features). Toluidine blue staining was also carried out for *H. pylori* density grading. A single blinded expert GI histopathologist carried out the histological grading. One biopsy from the antrum was placed in urease medium for rapid urease detection, and another sample in iso-sensitest broth (Oxoid, Cambridge, UK)/10% glycerol for *H. pylori* isolation and culture.

Statistical methods

Sensitivity, specificity, positive and negative likelihood ratios along with 95% confidence intervals (CI) for magnification endoscopy appearances and serological markers were compared with histology. A receiver operating characteristic (ROC) curve was used to assess the diagnostic accuracy. Chi-squared tests were used to compare the diagnostic accuracies. Kappa (k) statistics were calculated to determine inter-rater agreement among experts and non-experts. Interpretation of k values was as follows: <0= no agreement; 0–0.20= slight agreement; 0.21–0.40= fair agreement; 0.41–0.60= moderate agreement; 0.61–0.80= substantial agreement; and 0.81–1= almost perfect agreement (39). Differences in image quality were assessed using the Mann-Whitney U test. P values <0.05 were considered statistically significant. Stata version 14 (Stata Corporation, College Station, Texas) was used for the statistical analysis.

Results

Patient characteristics are described in Table 2. 24% of patients had histological evidence of *H. pylori* infection, 35% had gastric atrophy and 14.7% had intestinal metaplasia.

Diagnostic accuracy of NBI-Z and serology

NBI-Z diagnostic accuracy for determining normal corpus (Type I) was 0.87 (95% CI 0.82-0.92), *H. pylori* gastritis (Type II/III) 0.65 (95% CI 0.55-0.75) and gastric atrophy (Type IV) 0.88 (95% CI 0.81-0.94) respectively.

When NBI-Z was compared with serology, NBI-Z was superior for detecting gastric atrophy: NBI-Z Type IV 0.88 (95% CI 0.81-0.94) vs PG I/II ratio <3 0.74 (95% CI 0.62-0.85), p<0.0001 and NBI-Z 0.88 (95% CI 0.81-0.94) vs PG<30µg/l 0.75 (95% CI 0.64-0.87), p<0.0001. Although *H. pylori* IgG had a numerically higher accuracy this did not achieve statistical significance, NBI-Z Type II/III 0.65 (95% CI 0.55-0.75) vs *H. pylori* IgG 0.82 (0.73-0.9), p=0.078.

Diagnostic accuracy of NBI-Z and WLE-Z

Using the Nottingham classification, NBI-Z performed better than WLE-Z for detecting normal corpus and gastric atrophy. Normal corpus (Type I): NBI-Z 0.87 (95% CI 0.82-0.92) vs WLE-Z 0.82 (95% CI 0.76-0.87), p<0.0001. Atrophy (Type IV): NBI-Z 0.88 (95% CI 0.81-0.94) vs WLE-Z 0.81 (95% CI 0.73-0.89), p<0.0001. However, WLE-Z performed better than NBI-Z for detecting *H. pylori* gastritis: NBI-Z 0.65 (CI 0.55-0.75) vs 0.69 (95% CI 0.6-0.79), p<0.0001. (Table 3).

Diagnostic accuracy of the Modified Porto classification

NBI was more accurate than WLE for detecting normal mucosa, *H. pylori* gastritis and atrophy in the corpus. Normal corpus (Ab): NBI-Z 0.81 (95% CI 0.74-0.87) vs WLE-Z 0.79 (95% CI 0.72-0.87), p<0.0001. *H. pylori* gastritis (Ab+): NBI-Z 0.77 (95% CI 0.67-0.87) vs WLE-Z 0.62 (95% CI 0.51-0.74), p=0.01. Gastric atrophy (Db): NBI-Z 0.71 (95% CI 0.62-0.79) vs WLE-Z 0.65 (95% CI 0.56-0.73), p<0.0001. For detecting intestinal metaplasia, there was no statistical difference in accuracy. Intestinal metaplasia (Bb): NBI-Z 0.66 (95% CI 0.53-0.79) vs WLE-Z 0.59 (95% CI 0.45-0.73), p=0.28. (Table 4).

Inter-observer agreement

The mean kappa values for inter-observer agreement for NBI endoscopy images were higher among expert than non-experts endoscopists. For describing the corpus using the Nottingham classification the agreement was 0.63(95% CI 0.51-0.73) vs 0.5 (95% CI 0.39-0.62). When describing atrophy the agreement was 0.65 (95% CI 0.53-0.75) vs 0.47 (95% CI 0.2-0.68). Using the modified Porto classification when describing the corpus was 0.33 (95% CI 0.21-0.43) vs 0.2 (95% CI 0.12-0.28). When describing intestinal metaplasia the agreement was 0.36 (95% CI

0.24-0.47) vs 0.21 (95% CI 0.1-0.34). Both expert and non-expert endoscopists rated the overall image quality VAS higher for NBI than WLE (7 vs 6 p<0.0001).

Discussion

Standard WLE is limited in its ability to accurately diagnose gastric lesions, which has led to the development of a variety of techniques such as chromoendoscopy, NBI, flexible spectral imaging color enhancement (FICE), autofluorescence (AFI) and confocal laser endomicroscopy (19) in addition to gold standard histology. NBI has been extensively studied for its use in detecting dysplasia in the esophagus, bronchus and in colonic polyps (19, 40-42).

We conducted this pilot study to assess the role of NBI and serology in predicting the histological diagnosis of gastric mucosa. We demonstrated good correlation with normal and gastric atrophy using magnification endoscopy. However, for detecting *H. pylori* gastritis our results were limited with an accuracy of 0.65 using the Nottingham classification. Endoscopic diagnosis of *H. pylori* infection requires a subjective assessment of vascular density of subepithelial capillary network (SECN). A more objective and accurate measurement may be needed to increase accuracy. Overall, NBI-Z performed better than both WLE-Z and serological markers for predicting disease. This was the first study to compare NBI-Z and serological markers with gastric pathology.

The main factor influencing NBI appearance is light absorption by hemoglobin within blood vessels which in turn leads to variation in the vascular pattern of the gastric mucosa. The differences seen in gastric mucosa using NBI are a true reflection of the structural differences seen *in vitro* histologically. This in turn allows NBI to predict histological diagnosis (43). The endoscopic appearance of gastric atrophy has been established since the 1970's (44). The normal gastric body consists of a regular pattern of honeycomb type SECN and collecting venules but in *H. pylori* gastritis this is irregular and accompanied by surrounding edema (18, 45).

Very few studies have investigated the endoscopic appearance of *H. pylori* gastritis, with nodular mucosal and gastric fold hypertrophy the most consistent features despite low sensitivities when compared to histology (46, 47). Thus the exact features that describe *H. pylori* gastritis are not entirely known which makes description and interpretation difficult. The findings using the Nottingham classification were lower than when initially described using WLE (18). Potential

reasons for this include the fact that the principal endoscopists scoring the images did not perform the procedures and, therefore, were blinded to potential clinical information that could influence decision making. Although the modified Porto classification required more time to examine the images it also offered more details in terms of the presence of intestinal metaplasia and dysplasia. The specificity was high for normal mucosa, gastric atrophy and intestinal metaplasia. Potentially this could enable endoscopists to confidently avoid taking biopsies in the corpus. The evidence for the use magnified endoscopy in detecting other disease shows the majority have good correlation with histology (18, 28, 48-50). Currently, the gold standard for the diagnosis of intestinal metaplasia and dysplasia remains histology despite promising results with NBI.

When compared with serological markers, NBI-Z overall performed better. Previous studies have also shown that NBI is accurate in detecting premalignant lesions (29, 30, 51). The serological data presented in this study was similar to previous studies in terms of sensitivity and specificity (13, 31, 52, 53). *H. pylori* serology is commonly used in clinical practice in the UK but markers of atrophy are not. These results suggest the PG I/II ratio or PG I alone cannot replace endoscopy surveillance or detection of gastric atrophy. However, using this in clinical practice could reduce the need to obtain histology if serology is negative and NBI-Z does not suggest disease, therefore, reducing associated costs and time.

Inter-observer agreement was unsurprisingly higher among expert endoscopists when compared with non-experts. Endoscopy assessment is dependent on experience and training, so these techniques are likely to perform better in the hands of experts (54, 55). The training of western endoscopists is also likely to be different compared to Asian countries where the incidence of gastric cancer is higher. The observers VAS median scores were higher for NBI-Z than WLE-Z suggesting NBI provided more clarity to enable a diagnosis.

Our results are more applicable to specialist centers that routinely use NBI endoscopy as it requires a certain skill level and so may not always be practical to use in routine clinical practice. In the same way, the captured images in this study were captured by endoscopists with advanced diagnostic imaging experience and this is unlikely to happen routinely. As shown by the lower agreement amongst non-experts, NBI requires training in mucosal pattern recognition. The main comparison was done with experts so if this was used in a real world setting the results may be

poorer. Also the time duration each endoscopist spent reviewing the still images for the inter-observer study was not specified and unlike time constraints seen in clinical practice, possibly influencing the decision making process. In terms of training, this study did not measure inter-observer agreement before and after the training session which would have given some insight into the learning required for NBI use in a routine clinical setting.

Study strengths include the use of two endoscopy classification criteria to describe the magnified gastric mucosa in addition to serological markers in a large cohort of prospectively recruited patients. Post endoscopy image assessment controlled for clinician influence on pre-test probability. Bias was also reduced by blinding both the endoscopists and histopathologist to clinical data. Also the consistent use of the same gastroscope reduced image quality variability. Patients on proton pump inhibitors were also excluded to lower the number of false negatives in terms of *H. pylori* infection and to avoid missing early gastric neoplasia (56). This work has provided further evidence to support routinely investigating the presence of gastric atrophy and *H. pylori* gastritis in iron deficiency anemia patients (31, 36).

With regards to study limitations, by including only iron deficiency anemia patients this makes the study prone to selection bias and thus may not be representative at a population scale. However, as the annual incidence of gastric atrophy is low (0-10.9%) (57) we needed a larger cohort to enable estimates of sensitivity and specificity to be made to guide sample size calculations in future larger studies. Extent of disease also influenced the results. For example, atrophy serology tests only detect moderate to severe atrophy so endoscopy is more likely to perform better as these classifications only detect the presence of atrophy and not the degree. Inter-observer agreement for the modified Porto classification may also be lower due to the examiners been more familiar with the Nottingham classification at this specialist center. Finally, although the addition of atrophy to the modified Porto classification provided a more detailed description which more accurately resembled histology it also made the classification more complex and time consuming. The diagnosis of intestinal metaplasia is more reliable in terms of both histological grading and disease progression.

In conclusion, NBI-Z can detect changes in the GI mucosa which are usually dependent on histology. Although serology performs well, NBI endoscopy performs better in terms of disease detection with a high specificity and moderate to substantial observer agreement. A detailed

examination with NBI-Z could potentially help identify early precancerous lesions, which could enable patients to be promptly enrolled in appropriate endoscopic surveillance with improved disease outcomes. Also NBI-Z use may allow stratification of the need for histology and thus minimize associated costs, time and sampling error. These study findings will help to design future trials to evaluate NBI techniques in the gastric mucosa.

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Disclosure statement

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Figures

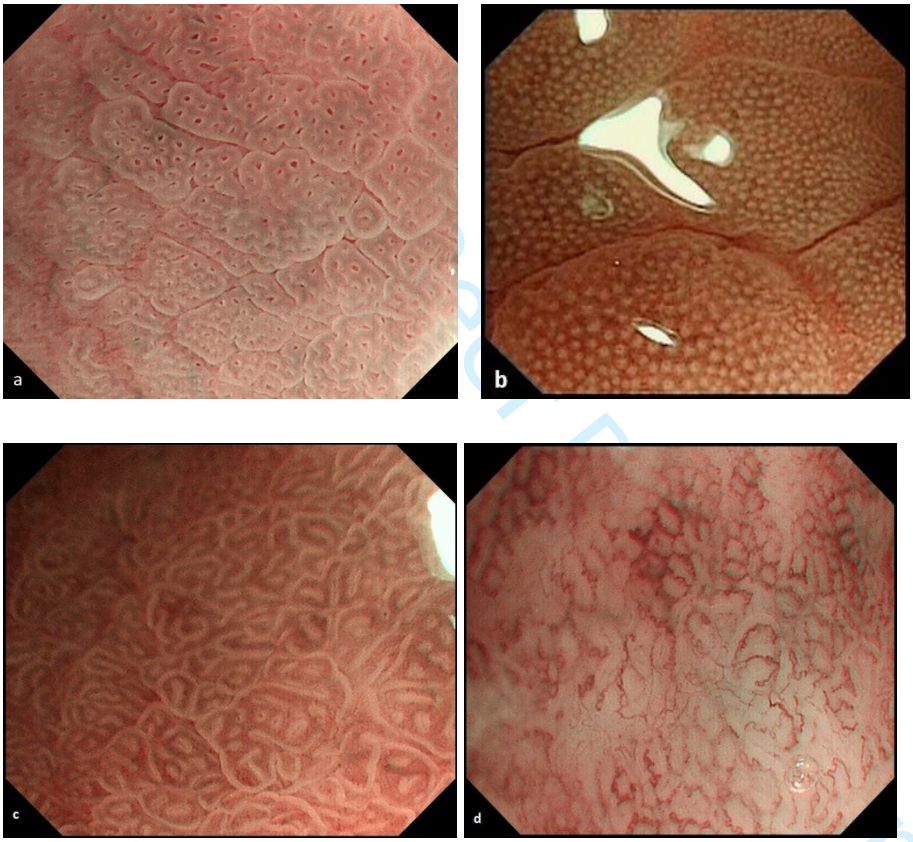
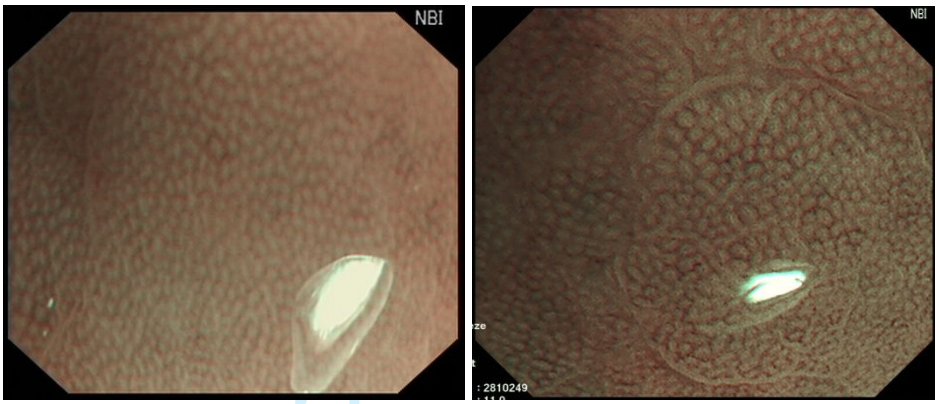
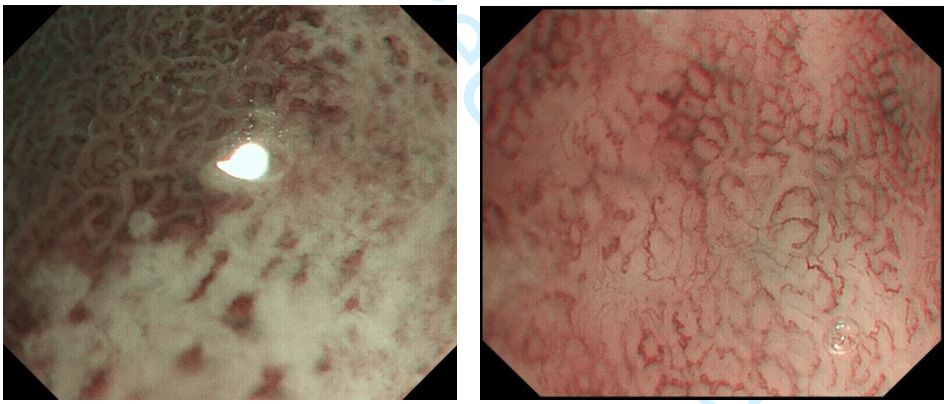


Figure 1 The Nottingham classification in gastric corpus

Type I (a) normal gastric body microvasculature = a honeycomb type subepithelial capillary network (SECN) and collecting venules in a regular arrangement. Type II (b) = honeycomb−type SECN with regular round pits, either with or without sulci, but with loss of collecting venules. Type III (c) = loss of the normal SECN and collecting venules, with enlarged white pits surrounded by erythema. Type IV (d) = loss of the normal SECN and round pits, with irregular arrangement of the collecting venules (18).



A B



C D

Figure 2 Modified Porto gastric corpus classification

Normal corpus (Ab) in image (A), (B) *H. pylori* gastritis corpus (Ab+), (intestinal metaplasia in corpus with some light blue crest (Bb) in image (C) and atrophy in corpus (Db) in image (D). Magnification 115X.

Tables

Table 1 Modified Porto classification

	A*	B*	*H. pylori*+	C*	D*
Mucosal pattern	Regular circular/oval	Regular Ridge/tubulo-villous with or without light blue crest	Regular	Irregular/ absent with or without white opaque substance	Absent
Vascular pattern (SECN)	Regular mucosa and SECN Vascular pattern central in antrum/peripheral in corpus of gland	Regular	Regular/ variable vascular density	Irregular	Absent
Expected outcome	Normal	Intestinal Metaplasia	*H. pylori* infection	Dysplasia	Atrophic gastritis

The letter (a) for antrum or (b) for corpus is added to the class type to signify the sites. For *H. pylori* positive pattern, the symbol (+) is added to end of the pattern class site. For e.g. *H. pylori* positive pattern in corpus would be classed as Ab+ (24).

Table 2 Patient description and laboratory data

Numbers	116
Male: Female ratio	1:1.1
Age, median (range) years	67 (19-85)
% Pharyngeal local anesthetic: conscious sedation	38%:62%
Midazolam median dose (range)	3mg (1-5)
Pethidine median dose (range)	25mg (25-100)
Hemoglobin males (130-180g/L) median (range)	110g/L (85 -129)
Hemoglobin females (120-165g/L) median (range)	101 g/L (42-118)
MCV (84-102fl) median (range)	83fl (56-111)
Ferritin (25-350µg/L) median (range)	12 µg/L (1-474)
Iron (9-31µmol/L) median (range)	11 µmol/L (1-83)
Histological diagnosis:	
% *H. pylori* infection	24%
% Atrophy corpus	35%
% Intestinal metaplasia	14.7%

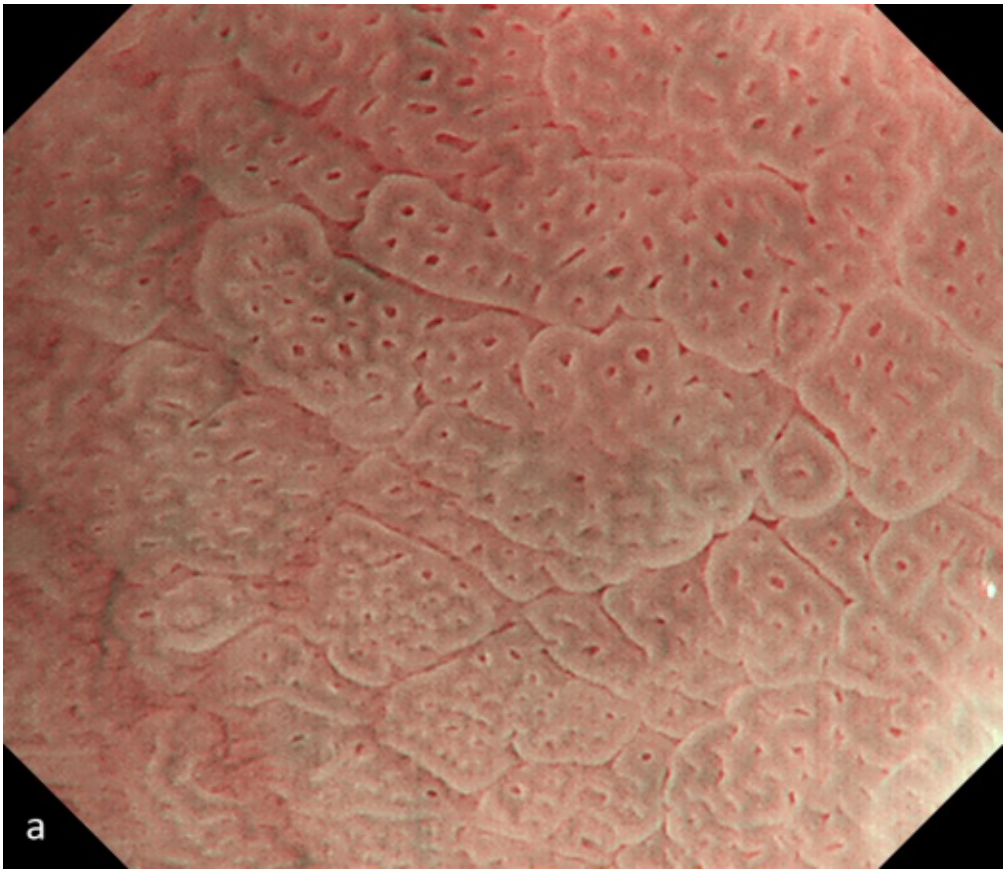
Presented as median with full range

Table 3 Sensitivity, specificity and likelihood ratios with 95% CI of NBI-Z and WLE-Z for detecting *H. pylori* gastritis and gastric atrophy using the Nottingham classification and serology

	Sensitivity (95% CI)	Specificity (95% CI)	Positive Likelihood ratio (95% CI)	Negative Likelihood ratio (95% CI)
NBI-Z Type I	75.6% (64.6-84.7)	98.6% (92.2-100)	54.0 (7.4-366.8)	0.25 (0.17-0.37)
WLE-Z Type I	66.2% (54.6-76.6)	96.8% (89-99.6)	20.7 (5.3-82.4)	0.35 (0.25-0.48)
NBI-Z Type II/III	62.1% (42.3-79.3)	68.1% (58.8-76.4)	1.95 (1.32-2.87)	0.56 (0.34-0.9)
WLE-Z Type II/III	74.1% (53.7-88.9)	64.9% (55-73.7)	2.11 (1.5-2.95)	0.40 (0.21-0.77)
NBI-Z Type IV	75.6% (59.7-87.6)	100% (96.6-100)	-	-
WLE-Z Type IV	64.1% (47.2-78.8)	98% (93-99.8)	32.1 (8-130)	0.37 (0.24-0.56)
H. pylori IgG	90% (68.3-98.8)	73.9% (61.9-83.7)	3.45 (2.26-5.27)	0.14 (0.04-0.51)
PG I/II ratio <3	73.7% (48.8-90.9)	73.8% (60.9-84.2)	2.81 (1.71-4.63)	0.36 (0.17-0.77)
PG I <30µg/l	52.6% (28.9-75.6)	98.4% (91.2-100)	32.11 (4.4-235)	0.48 (0.3-0.77)

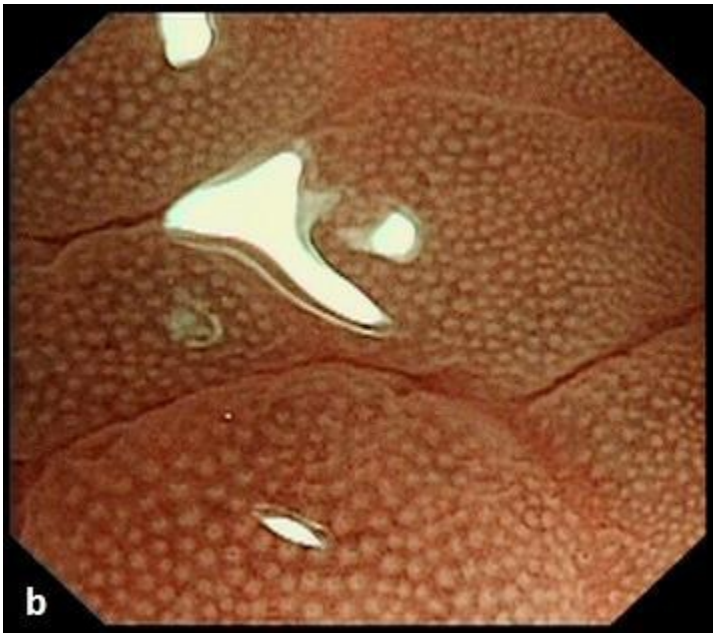
Table 4 Sensitivity, specificity and likelihood ratios with 95% confidence intervals of NBI-Z and WLE-Z using the modified Porto classification in the corpus

	Sensitivity (95% CI)	Specificity (95% CI)	Positive Likelihood ratio (95% CI)	Negative Likelihood ratio (95% CI)
NBI-Z Ab	70.5% (59.8-79.7)	90.9% (78.3-97.5)	7.75 (3-19.9)	0.32 (0.23-0.45)
WLE-Z Ab	77.8% (66.4-86.7)	81% (65.9-91.4)	4.09 (2.2-7.7)	0.27 (0.17-0.43)
NBI-Z Ab+	75% (53-90.2)	78.7% (69.8-86)	3.52 (2.3-5.4)	0.32 (0.16-0.64)
WLE-Z Ab+	43.5% (23.2-65.5)	81.3% (71.8-88.7)	2.33 (1.24-4.4)	0.69 (0.48-1.01)
NBI-Z Bb	35.7% (12.8-64.9)	95.8% (90.4-98.6)	8.43 (2.78-25.5)	0.67 (0.45-0.99)
WLE-Z Bb	27.3% (6-61)	90.1% (82.5-95.1)	2.75 (0.89-8.53)	0.81 (0.56-1.17)
NBI-Z Db	70% (52-81)	99% (94.5-100)	70.0 (10.74-307)	0.30 (0.23-0.78)
WLE-Z Db	61% (45-76)	100% (95.7-100)	-	0.39 (0.27-0.50)



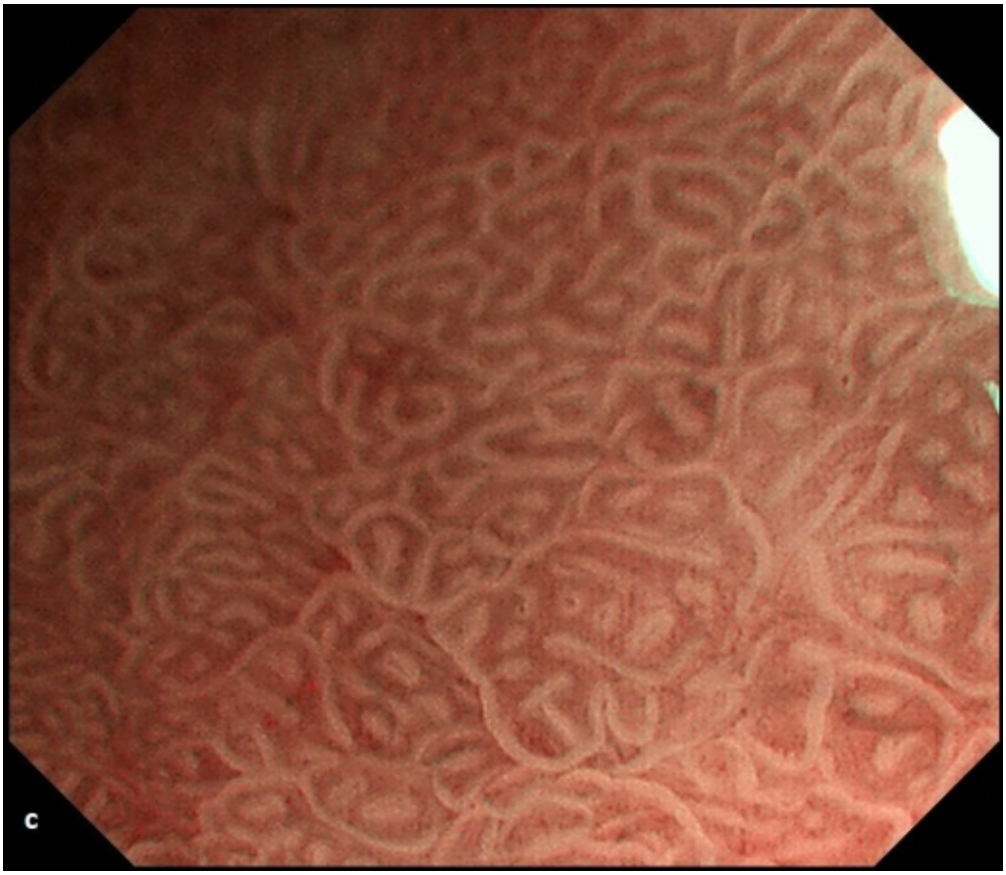
Type I (a) normal gastric body microvasculature = a honeycomb type subepithelial capillary network (SECN) and collecting venules in a regular arrangement.

60x52mm (220 x 220 DPI)



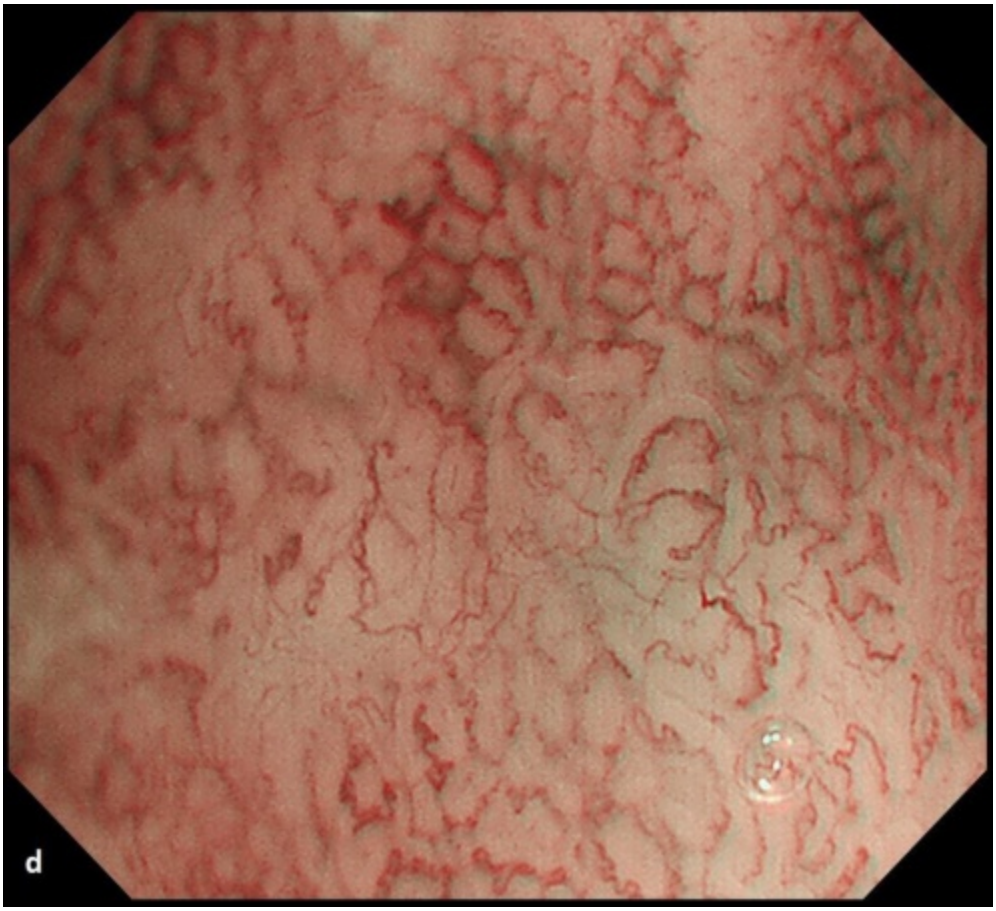
Type II (b) = honeycomb−type SECN with regular round pits, either with or without sulci, but with loss of collecting venules.

93x83mm (96 x 96 DPI)



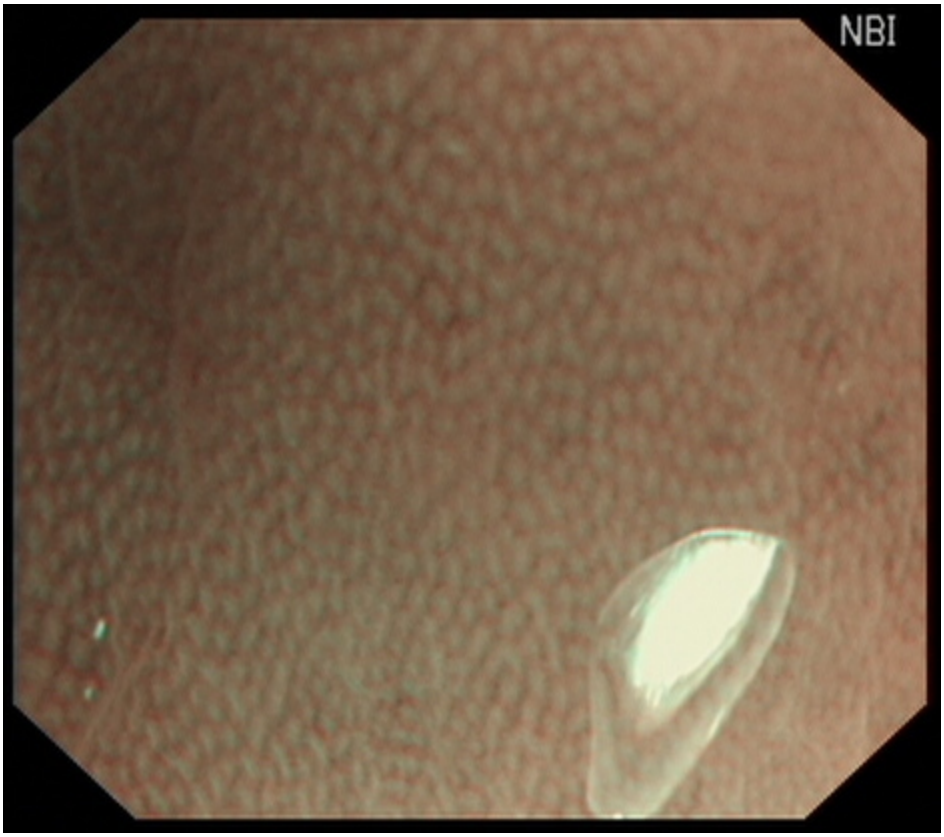
Type III (c) = loss of the normal SECN and collecting venules, with enlarged white pits surrounded by erythema.

59x51mm (220 x 220 DPI)



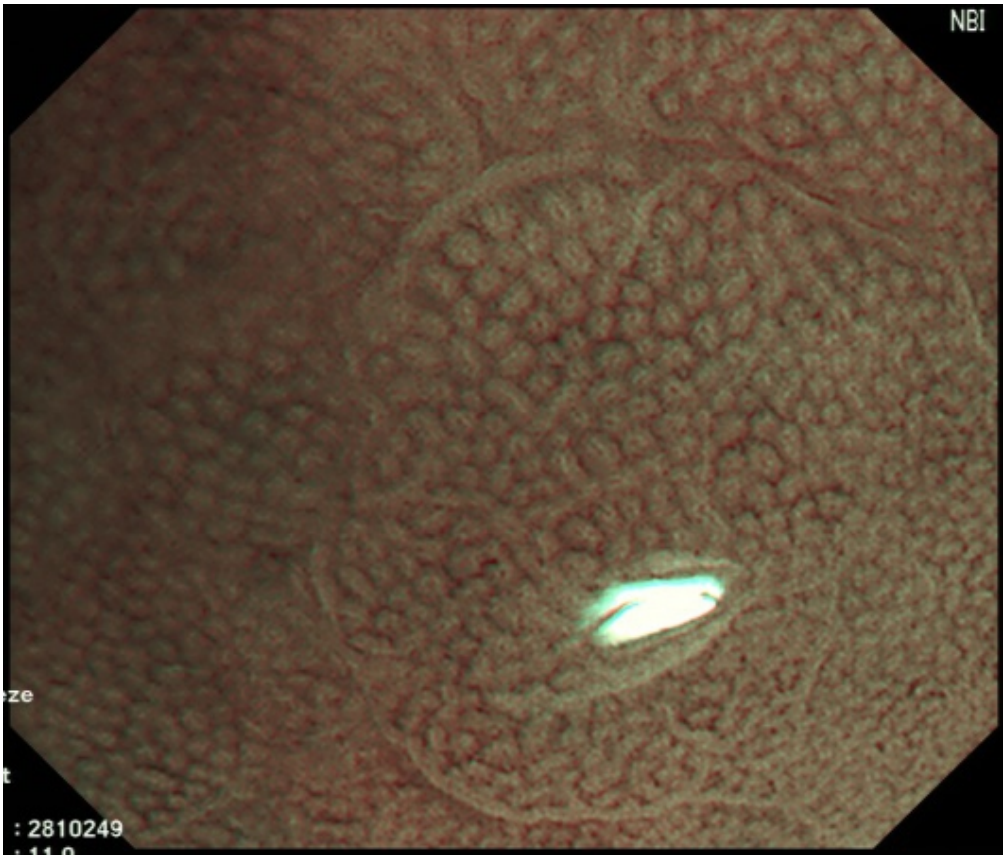
Type IV (d) = loss of the normal SECN and round pits, with irregular arrangement of the collecting venules (18).

57x52mm (220 x 220 DPI)



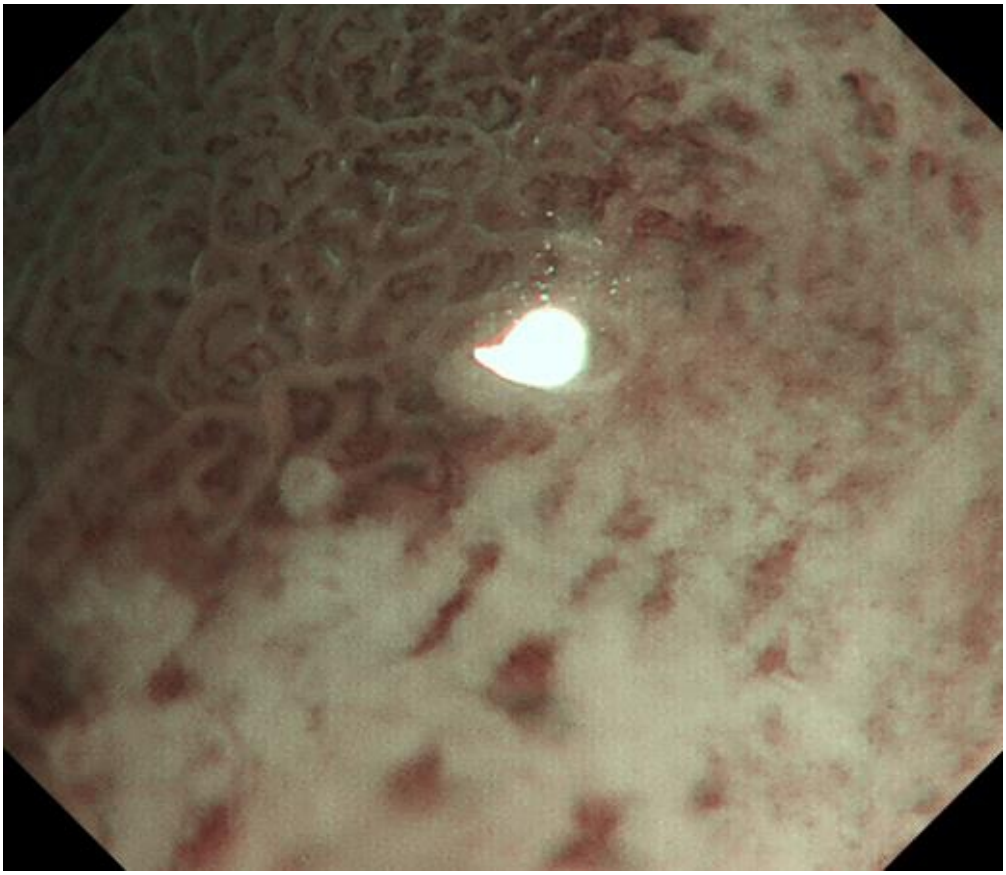
Normal corpus (Ab) in image (A)

165x146mm (72 x 72 DPI)



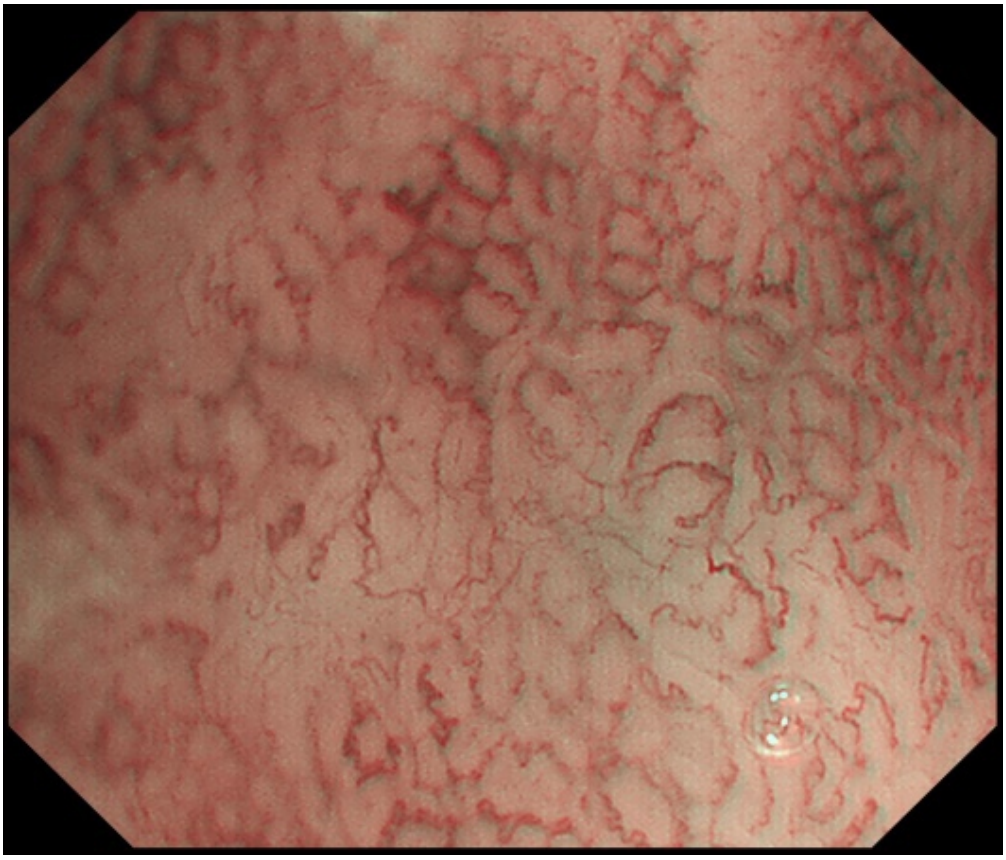
(B) H. pylori gastritis corpus (Ab+)

61x51mm (220 x 220 DPI)



Intestinal metaplasia in corpus with some light blue crest (Bb) in image (C)

59x51mm (220 x 220 DPI)



Atrophy in corpus (Db) in image (D). Magnification 115X.

61x51mm (220 x 220 DPI)