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Title: The value of indigo carmine staining in endoscopy and endoscopic mucosal resection in the human stomach

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The **history of the University of Szeged** dates back to 1581 when István Báthory, the Prince of Transylvania founded a higher education institution in Kolozsvár (Cluj-Napoca) which became prestigious in a short time. Due to its professors well-known all around Europe it provided high standard education and also had the right to confer baccalaureate and master's degrees. Moreover, it was the only institute for higher education in Hungary at the end of 16th century. Later Maria Theresia entrusted the Piarists to reorganize the institution as a result of which the Faculty of Medicine-Surgery was established in 1775. Later on, these served as the basis for the Hungarian Royal University of Kolozsvár, founded by Francis Joseph I in 1872. It was renamed after the king in 1881 and bore his name until 1940. The institution moved to Szeged in 1921.

Nowadays, the **Faculty of Medicine**, University of Szeged is one of the most outstanding medical schools in Hungary teaching health sciences in three languages. The Faculty has excellent scientific laboratories performing high standard researches supported by national and international grants. Students have a wide range of opportunities to join scientific research activities during the time of their studies. Experience gained during university years help many students to become successful researchers all around the world. The Faculty has four **Ph.D. Doctoral Schools** in which more than a hundred supervisors offer dissertation topic proposals. Notably, annually there are approximately 40 defended Ph.D. dissertations and graduations. It gained high reputation in research, education and practice of medical sciences. We are all proud of **Albert Szent-Györgyi**, former professor and dean of the Faculty who was awarded **Nobel Prize in 1937** for his research in Szeged. He is an idol both for lecturers and students, presenting the idea that world-wide results can be achieved in Hungary and Szeged.

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THE VALUE OF INDIGO CARMINE STAINING IN ENDOSCOPY AND ENDOSCOPIC MUCOSAL RESECTION IN THE HUMAN STOMACH

Ph.D. Thesis

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2008

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Abbreviations

GI - gastrointestinal

CE - chromoendoscopy

EMR - endoscopic mucosal resection

EGC - early gastric cancer

ESD - endoscopic submucosal dissection

List of full papers cited in the Thesis

I. Szalóki T: A korai gyomorrák néhány problémája. Új lehetőség a gastroenterologiában: endoscopos mucosa resectio. In. *A daganetellenes kűzdelem Pest Megyében* (szerk: Iglói F.) Budapest, 9-13, 1995.

II. Czakó L, Szalóki T, Lonovics J: A japán endoszkópos technika. *Magyar Belorvosi Archivum,* 2002; **55**: 29-35.

III. Szalóki T: Indigókarmin festékkontraszt módszer alkalmazása nagy felbontóképességű elektronikus endoszkópia során. *Orv Hetil* 2002; **143**: 25-29.

IV. Szalóki T, Tóth V, Czakó L: Unusual esophageal manifestations of Crohn's disease. *Gastrointest Endosc* 2006; **64**: 121.

V. Szalóki T, Tóth V, Tiszlavicz L, Czakó L: Flat gastric polyps: Results of forceps biopsy, endoscopical mucosal resection, and long-term follow-up. *Scand J Gastroenterol* 2006; **41**: 1105-1109.

VI. Szalóki T, Tóth V, Tiszlavicz L, Czakó L: A gyomor endoszkópos mucosaresectiója-diagnosztikus és terápiás módszer. *Orv Hetil* 2006; **147**: 501-507.

VII. Szalóki T, Tóth V, Németh I, Tiszlavicz L, Lonovics J, Czakó L: Endoscopic mucosal resection: not only therapeutic, but a diagnostic procedure for sessile gastric polyps. *J. Gastroenterol Hepatol* 2007 Dec 5 [Epub ahead of print].

VIII. Szalóki T, Czakó L, Tóth V: Diagnosis and treatment of superficial neoplastic lesions of the stomach by endoscopic mucosal resection. *In. Gastric Cancer Research Trends (ed: Tompkins M.B.) Nova Science Publishers.* New York. 2008 (in press).

List of abstracts related to the subject of the Thesis

I. Szalóki T: A korai gyomorrák diagnosztikájának néhány problémája. Magyar Gasztro-enterológiai Társaság, Endoszkópos Szekcióülés, Győr, *Előadáskivonatok*, 1995. 3.

II. Szalóki T, Liebhardt J, Molnár Cs: Administration of indigo carmine dye contrast method to gastrointestinal endoscopy. Magyar Gasztroenterológiai Társaság 38. Nagygyűlése, *Előadáskivonatok*, 1996. 208.

III. Szalóki T, Tóth B, Varga Sz: Endoscopic mucosal resection for stomach mucosal lesions. *Z. Gastroent*. 1997; **35**, 403.

IV. Szalóki T: Indigocarmin festékkontraszt módszer alkalmazása nagy felbontóképességű elektronikus endoscopia során. *Magyar Belorvosi Archivum*, 1998/3; **Suppl**. 304,

V. Szalóki T, Károlyi P, Czirják I: It is possible to separate small adenomatous polyps from non adenomatous with high resolution colonoscopy and chromoendoscopy. *Z Gastroenterol* 1999; **37**, 447.

VI. Szalóki T, Tóth V, Liebhardt J, Molnár C, Rádics Z, Szász M, Lonovics J, Czakó L: Diagnostic and therapeutic use of endoscopic mucosal resection (EMR). *Z Gastroenterol* 2005; **43**, 477-529 A:127

VII. Szalóki T, Tóth V, Sükösd F, Lonovics J, Czakó L: The prevalence of Helicobacter pylori infection in hyperplastic polyps, adenomas and cancers of the stomach. *Z Gastroenterol* 2006; **44:** 411-458 A:123.

Summary

Introduction: Chromoendoscopy (**CE**) is an old endoscopic technique which is undergoing a renaissance in the era of high-resolution electronic endoscopy. It can improve the endoscopic diagnosis as a safe, simple, quick, widely available, inexpensive method, it requires minimal equipment, and most of the reagents are generally available. Indigo carmine, a deep-blue stain, is not absorbed by the gastrointestinal (**GI**) epithelium. Histological examination of specimens obtained by the forceps biopsy sampling of gastric lesions is of only limited accuracy, and their management on this basis is therefore controversial. Endoscopic mucosal resection (**EMR**) was initially developed in Japan for the resection of early gastric cancer (**EGC**). The potential use of EMR as a diagnostic tool has been suggested. The aims of this study were to assess the value of CE in the diagnosis of flat polypoid lesions and during EMR procedures, the value of forceps biopsy sampling in establishing the correct diagnosis revealed by EMR, and the efficacy of EMR.

Patients and methods:

CE: After a usual endoscopic procedure, the lesion or the locus of interest was identified. 0.1-0.5 % indigo carmine solution was administered to the gastric, duodenal or colonic mucosa. CE was used in all cases of EMR, and before polypectomies in the upper and lower GI tract. The dignity of the flat polypoid lesions < 1 cm in diameter in the colon was predicted.

EMR: 56 subjects with sessile gastric polyps of epithelial origin, at least 0.5 cm in diameter, were included. Following forceps biopsy sampling, EMR was performed with an inject-and-cut technique or with cap-fitted methods. The histological results on the forceps biopsy and the resected specimens were analyzed.

Results:

CE: 3 times as many gastric adenomas were diagnosed when CE was applied. In the colon, the surface appearance of colonic crypts was studied, and CE promoted the discrimination between hyperplastic polyps, which had a typical ”pit” pattern (”dots”), and adenomatous polyps, which had a ”groove” or ”sulci” pattern. The diagnostic accuracy proved to be 59/64 (92%) in the adenomatous polyps, and 18/23 (78%) in the hyperplastic polyps, as revealed by histological examination.

EMR: Histology on the resected specimens revealed neoplastic lesions in 34 cases, including 7 EGCs, and there were hyperplastic-inflammatory lesions in 21 cases. Complete agreement between the previous histological results on the forceps biopsy samples and the resected

specimens was seen in only 55.3% of the lesions. Overall, the sensitivity and specificity of the forceps biopsy procedure for diagnosing neoplastic lesions were 87.5% and 83.3%, respectively. A clinically relevant discrimination between neoplastic and non-neoplastic lesions was not achieved in 7 cases. In 14 neoplastic and 1 hyperplastic polyps, the degrees of dysplasia seen on histological examination of the forceps biopsies and the resected specimens were different. In 4 cases, foci of carcinoma were present in the resected specimens that were missed by biopsy sampling. We observed 6 (10.7%) recurrences during the follow-up period. None of the EGCs recurred during the mean 38-month (6-72) follow-up. No complications such as perforation or massive bleeding necessitating surgical treatment were encountered.

Conclusions:

CE: The contrast staining with indigo carmine highlighted and delineated irregularities in the mucosal architecture. The results could be evaluated immediately after application of the dye. We found that CE furnished a dramatic accentuation of abnormalities of the mucosal architecture and it was possible to discriminate various pit patterns.

EMR: Forceps biopsy is not fully representative of the entire lesion, and a simple biopsy may therefore lead to a faulty differentiation between neoplastic and non-neoplastic lesions. EMR offers diagnostic and staging advantages in assessing patients with EGC as compared with forceps biopsy, because it provides more intact mucosa and submucosa for histological analysis. Sessile gastric polyps should be fully resected by EMR for a final diagnosis and (depending on the lesion size and type) possibly definitive treatment.

1. Introduction

A concerns its incidence, gastric cancer is currently the fourth leading cancer type (after lung, breast and colorectal cancers), accounting for 8.6% of all cases of cancer. 934 000 new gastric cancer cases were diagnosed worldwide in 2002 (1). The prognosis of gastric cancer is better than it was earlier in Europe and the USA, but it is still far from that achieved among the Japanese (2). A recent review from the National Cancer Data Base revealed that the 5-year and 10-year survival rates of patients diagnosed with gastric cancer in the USA between 1985 and 1996 were 28% and 20%, respectively (3). In contrast, the 5-year survival rate of gastric cancer in Japan is 50% (4). Indeed, 70% of gastric cancer patients detected by screening and treated by gastric resection in Japan are still alive after 5 years postoperatively.

Early-stage gastric cancer (**EGC**) is defined as the state when the tumor invasion is confined to the mucosa or submucosa, irrespective of the presence or not of lymph node metastasis (5). The incidence of EGC detection is nearly 60% in Japan, close to 0% in Hungary and not more than 10% in other developed countries (1). The 5-year survival rate of EGC in Japan is 96-99% (6).

The aim of endoscopic mucosal resection (**EMR**) is the complete removal of diseased mucosa by resection through the middle or deeper part of the submucosal layer (7). EMR of superficial early cancers of the upper gastrointestinal tract (**GI**) is a standard technique in Japan and is currently increasingly used in other developed countries. EMR is a therapeutic procedure that allows the removal and retrieval of tissues for pathological examination. Detailed pathological examination of the resected tissues may permit refinement of the diagnosis made via the histological examination of forceps biopsy samples, and the curability or the need for additional surgery can be evaluated more precisely. EMR with a proper indication can furnish a cure rate equivalent to that of surgical resection (7).

The Eastern-European and Hungarian results relating to EMR are essentially anecdotal, based on very low numbers of patients, short-term experience, and the non-routine use of intravital staining methods and magnifying endoscopies (8-11). There have been no long-term follow-up studies regarding EMR in Europe or the USA (12).

Necessity of early diagnosis

It is clearly necessary to improve the early diagnostic tools, including chromoendoscopy (**CE**), and to understand the potential role of EMR in the management of gastric polyps and in the demonstration of adenomas with various dysplasias.

The endoscopic appearance is an unreliable predictor of polyp histology. Forceps biopsy sampling often yields tissue that is inadequate for a correct histological diagnosis and the foci of dysplasia may not be identified (13-15). The technique of EMR allows removal of the entire lesion, which is then available for thorough histological examination and precise tumor staging.

New endoscopic resection techniques, called endoscopic submucosal dissection (**ESD**), allow the resection of larger lesions *en bloc*; the long-term data for this method are currently being assessed. Although ESD requires advanced endoscopic technical skills and involves a longer procedure time, this type of resection rapidly became popular in Japan, primarily because of the ability to remove large lesions *en bloc* and the possibilities of more precise histological staging and the prevention of disease recurrence (16, 17).

1.1.Chromoendoscopy

CE is a technique in which stains are applied topically to the GI mucosa in conjunction with endoscopy in order to improve localization, characterization or diagnosis. There is no uniform terminology for the different CE methods. For endoscopic GI mucosal staining, the terms chromoendoscopy, vital staining, contrast endoscopy, chromoscopy, endoscopic dye spraying, and endoscopic dye scattering are used alternatively.

CE is an endoscopic technique that has been utilized for several decades. In 1973, Ida *et al*. first published an article concerning a staining method for gastroscopy (18). In recent years, there has been a renaissance of CE as an endoscopic technique that can improve the endoscopic diagnosis as a safe, simple, quick, widely available, inexpensive method. Vital staining has become even more relevant because of endoscopic therapies which require the sophisticated selection of patients suitable for EMR, ESD, photodynamic therapy, multipolar electrocoagulation, argon plasma coagulation, *etc*.

CE requires minimal equipment and most of the reagents are generally available. Staining can be performed with any kind of endoscope and stains are usually applied onto the mucosa directly via a syringe through the biopsy channel of the endoscope or via a spray catheter. Some investigators emphasize that it is essential to delivery the stain to the mucosa in a fine mist (19).

No specific mucosal preparations are necessary for most of the methods. In order to obtain a good quality of staining, surface mucus, blood or food should be removed by using a water

flush. Sedation and administration of an antimotility drug are not prerequisites for CE, but could be of benefit in the planning of endotherapy after dye staining.

The interpretation of CE results needs to be learned and requires an understanding of what types of tissue are stained and what are not; it demands both positive and negative staining features. The various agents used for CE are categorized into four groups: absorptive stains, reactive stains, contrast stains and agents for tatooing (20-22) (Table 1).

1.1.2. Absorptive stains

Absorptive stains (*e.g*. Lugol's solution, toluidine blue, cresyl violet and methylene blue) are absorbed by the GI epithelium, allowing the identification of specific epithelial cell types or cellular constituents.

1.1.3. Reactive stains

Reactive stains (*e.g.* Congo red and phenol red) identify cellular products, through a color change of a pH indicator, and highlight acid-secreting or alkaline-associated epithelia (23, 24).

1.1.4. Tattooing agents

Tattooing is a means of permanently labeling a site in the GI tract by an intramural injection of a pigment for future identification. A major concern for tattooing was the desire to facilitate, both for the surgeon and for the pathologist, the localization of a histologically confirmed but otherwise readily overlooked carcinomatous or precancerous lesion during EMR, during surgery, or in a resected specimen. A number of dyes have been employed for tattooing, including Evans blue, Patent blue, methylene blue, toluidine blue, indigo carmine, indocyanine green, Lymphazurin, hematoxylin-eosin and India ink. For tattooing, the most often used is India ink, which consists of inert carbon particles suspended in aqueous or nonaqueous stabilizers and diluents. The dilution and technique of India ink tattooing is highly variable; the preferred methods for tattooing involve sterilization by autoclaving or gas sterilization. The tattoo is injected through a sclerotherapy needle directly into the submucosa.

1.1.5. Contrast stains

Contrast stains are not absorbed and do not react with the surface mucosa, but accumulate in the mucosal depressions and highlight the tissue topography of the mucosal surface.

Indigo carmine (disodium 3,3'-dioxo-2,2'-bis-indolylidene-5,5'-disulfonate) is a blue contrast stain, derived from the blue plant dye (indigo) and a red coloring agent (carmine) formed from cochineals by the addition of alum.

This stain is not absorbed by the GI epithelium. It pools in crevices between the epithelial cells, highlights small or flat lesions and defines irregularities in the mucosal architecture.

In the stomach, indigo carmine can be used to diagnose small gastric cancers;

in the duodenum, it has been used to evaluate villous atrophy in patients suspected of having malabsorption from celiac disease or tropical sprue (25).

In the colon, it has been applied to study the surface appearance of colonic crypts and to discriminate between hyperplastic polyps which have a typical pit pattern and adenomatous polyps which have a groove or sulci pattern (26). It can also aid in the diagnosis of minute, flat or depressed colorectal tumors (27, 28).

1.2. Endoscopic mucosal resection

Principles and indications

Several Japanese series indicate that the surgical resection of EGCs offers an excellent (90-100%) chance of a cure (29). Any surgical intervention involves the risk of complications, including death, anesthetic complications, wound infection and a prolonged hospital stay. This is an especially severe problem in patients with concomitant diseases, including lung and heart disease, kidney failure and diabetes or in elderly patients. Endoscopic resection offers an efficacy similar to that of surgery, it is minimally invasive and cheaper to perform (30, 31). EMR for EGC was first performed in Japan (32, 33). Endoscopic resection allows complete pathological staging of the cancer, which is critical as it allows stratification and the refinement of further treatment (34). Because of the low incidence of lymph nodes and distant metastases, surgical resection of the stomach for EGC is considered unnecessary and EMR is therefore more appealing.

According to data from the Cancer Institute Hospital in Tokyo, type IIc mucosal cancer < 10 mm in diameter carries no risk of lymph node involvement; type IIc mucosal cancer < 20 mm in diameter has a lymph node involvement rate of 0.4%; type IIa mucosal cancer < 20 mm in diameter has no risk of lymph node metastasis; type IIa submucosal cancer < 20 mm in diameter likewise has no risk of lymph node involvement; but submucosal cancer < 30 mm in diameter has a lymph node involvement rate of 28.6% (35).

The indications for EMR for EGCs over the past 20 years, since the introduction of the technique, have generally been accepted to include differentiated mucosal adenocarcinoma, elevated-type mucosal cancer < 20 mm in largest diameter, and a flat or depressed-type lesion (without ulceration) < 10 mm in diameter. If all these criteria are met, and if no lymphatic involvement of the tumor is noted on histological evaluation, the incidence of lymph node involvement is < 0.4% (36). The rationale behind this recommendation is that larger lesions or lesions with diffuse histology type may extend into the submucosal layer and have a higher risk of lymph node metastasis. More recently, however, using a large database involving 5265 patients who underwent gastrectomy with meticulous R2 level lymph node dissection, Gotoda *et al*. were able to define the risks of lymph node metastasis in additional groups of patients with EGC with increased certainty (37). None of the 1230 well or moderately differentiated intramucosal cancers < 30 mm in diameter, regardless of the ulceration findings, were associated with metastases. None of the 929 lesions without ulceration were associated with nodal metastases, irrespective of the tumor size. None of the 145 differentiated adenocarcinomas with minute submucosal penetration (sm1) and tumors size < 30 mm in diameter, without lymphatic or venous permeation, were associated with lymph node metastasis. There was no lymph node metastasis of the 256 undifferentiated (poorly differentiated adenocarcinoma or signet-ring cell carcinoma) intramucosal cancers without ulcer findings and < 20 mm in size, with no lymphatic vessel invasion. There was a significant correlation between a tumor size larger > 30 mm and lymphatic–vascular involvement with an increased risk of lymph node metastasis. The results of this study allowed the development of expanded indications for EMR, but the resection of larger lesions was not technically feasible until the development of ESD techniques (38).

1.2.1. Different endoscopic mucosal resection techniques

Several different EMR techniques have been described, but basically there are two groups of EMR techniques in the stomach: without suction or with suction (Fig. 1).

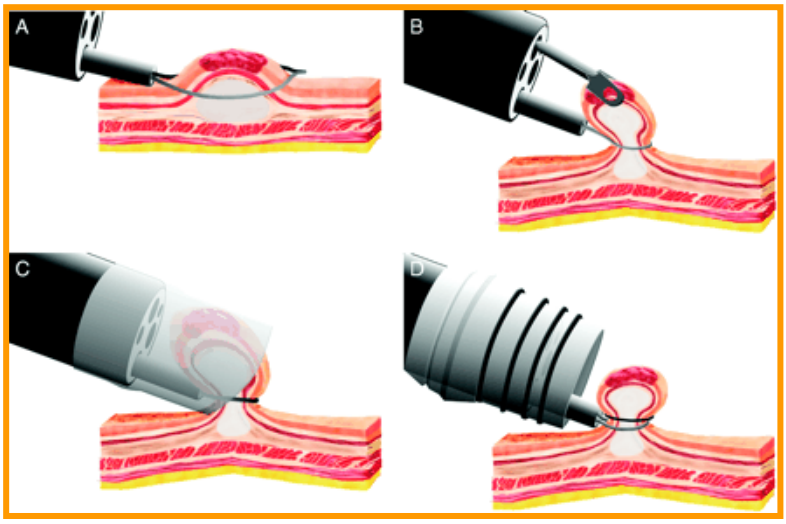

Fig. 1.

Different EMR techniques:
A: strip-off biopsy (injection and snaring)
B: lift and cut biopsy (grasping and snaring with double channel endoscope)
C: EMR-C: EMR using a transparent plastic cap (injection and snaring using a cap)
D: EMR-L: EMR using a ligating device (endoscopic ligation and snaring)
(Fig. from American Society of Clinical Oncology)

Without suction:

(a) strip-off biopsy (injection and snaring) (39).

The lesion is lifted with a submucosal injection, then snared and resected.

(b) Lift-and-cut biopsy (grasping and snaring with a double-channel endoscope)

Lift-and-cut biopsy requires the use of a two-channel endoscope. A snare and forceps are introduced through each channel of the endoscope. The forceps are used to guide the lesion into the snare, which is then closed around the base of the lesion and resection is performed with standard electrocautery (32). This technique was reported by Martin in 1976 (40).

Takekoshi recommended a method for retracting the mucosa with a grasper and then strangulating it with a snare (41).

With suction:

(a) EMR-C: EMR using a transparent plastic cap (injection and snaring using a cap)

A transparent cap with a prelooped snare on its distal tip is placed on the end of an endoscope. The lesion is lifted with a submucosal injection in the standard fashion. Suction is then used to draw the lesion into the cap, and the snare is closed on the base. It is next released from the cap by breaking the suction, and removed similarly to any polypoid lesion. Thus, the resection can be safely performed through the submucosal layer under the lesion (33).

Different-sized soft or hard caps are available according to the diameter of the endoscope and the size of the target lesions (42).

(b) EMR-L: EMR using a ligating device (endoscopic ligation and snaring)

The technique of EMR-L uses the standard endoscopic variceal ligation device to capture the lesion and make it into a polypoid lesion by deploying the band underneath it. A snare is then used to resect the lesion, usually below the level of the rubber band. Previously, the lesion may be injected submucosally for lifting (43, 44).

These EMR techniques with suction have the advantage of being relatively simple, use a standard endoscope and do not require an additional assistant. EMR-C and EMR-L are suitable for lesions not > 15 mm in one piece, but piecemeal resections can cause the pathologist to carry out pathological staging with some uncertainty, and there is a high risk of recurrence after piecemeal resections (45). Resection of larger lesions is more feasible by ESD.

European experience with EMR, especially for EGCs, is still relatively limited, since EGC is diagnosed at a much lower rate in Europe than in Japan, and operable patients are generally referred to surgery for radical resection. There have been no long-term follow-up studies regarding EMR in Europe and there are no data relating to EMR from Eastern Europe.

This thesis reports 10-year experience with the endoscopic management of flat gastric polyps, examining the correlation of the histological examination of the specimens obtained by forceps biopsy with the final diagnosis revealed by EMR.

2. Materials and Methods

2.1. Chromoendoscopy in the stomach

I learnt CE and endoscopic mucosal resection from a Japanese expert in the Gastroenterology Unit of Toranomon Hospital in Tokyo. The CE and EMR were performed in Japanese style (46). The application of CE and EMR was approved by the ethics committee

at Ödön Javorszky Hospital and the First Department of Medicine at the University of Szeged. After approval, indigo carmine powder made by Reanal Hungary was used. From 1994 to 1996, fiberoptic endoscopes, and from 1997, high-resolution video-endoscopes with 410 000 pixels and the possibility of magnification (Fujinon EG-140 HR, ED-140 XU and EC-410 HM) were used in our endoscopy rooms.

After the usual esophago-gastro-duodenoscopic procedure until the descending part of the duodenum, the lesion or the locus of interest was identified. The lesion and the surrounding mucosa were washed through the biopsy channel of the endoscope with 10-40 ml tap water and a 0.1-0.5% solution of indigo carmine was applied through the biopsy channel too. The amount was 3-10 ml. Rarely, a spray catheter (Olympus PW-5L) was used to make a fine mist for the mucosa. In patients with a resected stomach, 160 mg dimethicon (Ceolat, EGIS or Espumisan L emulsion, Berlin-Chemie) was applied 30-90 minutes before upper GI endoscopy for antifoaming. We have never used mucolytic agents. The indigo carmine staining method was used in 70% of total upper GI endoscopies in cases of: suspected malignancy, gastric ulcers, polyps, erosions, suspected gastric metaplastic islands, suspected chronic gastritis, operated stomach, Barrett's esophagus, celiac sprue or suspected malabsorption. Indigo carmine staining was used in all cases of EMR, and before polypectomies in the upper and lower GI tract.

The diagnosis of small (< 1 cm in diameter) gastric adenomas is difficult. From 1993 to 1996, when we were using fiberoptic gastroduodenoscopes we were looking for pale, demarcated flat lesions. During that period, 3680 patients were examined. From 1997 to 2000, 3782 patients were examined and 410 000 pixels electronic high-resolution videogastroscopes were used with indigo carmine contrast staining. At closer views (from 2-3 mm to the mucosa), we could achieve 30-50x magnification, which was not possible for the human eye previously.

2.2. Chromoendoscopy in the colon

During coloscopies, I endeavoured to reach the cecum or terminal ileum. Indigo carmine was used after a tap water flush to clean the mucosal surface or the lesion. 3-10 ml or more of 0.1-0.5% solution was applied as necessary. The main indications of indigo carmine staining in the colon were: minute tumors, flat lesions or flat adenomas, laterally spreading tumors, non- stenotizing tumors, polyps, ulcus simplex recti, patients after colon surgery

(anastomoses), ulcerative colitis (areas of suspected malignancy) and mild mucosal irregularities.

During colonoscopies from 1998 to 1999, using a high-resolution electronic coloscope and indigo carmine contrast staining, I tried to predict the dignity of flat polypoid lesions < 1 cm in diameter. The method was previously described by Axelrad (47) and Carr-Locke and Benjamin (48). The pit pattern (the features of crypts of Lieberkühn) was divided into two groups: the ”dotted” pattern was characteristic for hyperplastic, and the ”groove or sulci” (gyrus-like) pattern for adenomatous polyps. All these polyps were removed by simple snare polypectomy or by EMR. The pathologists were blinded regarding these groups. The aim was to establish how this simple method promotes the diagnostic accuracy of endoscopy.

2.3. Endoscopic mucosal resection in practice

Between 1994 and 2004, a prospective bicenter study was conducted in Jávorszky Hospital, Vác, and the First Department of Medicine at the University of Szeged. Gastric polyps of epithelial origin, at least 0.5 cm in diameter, were included in the study. Fundic glands and polyp cases associated with polyposis syndromes were excluded.

High-resolution electronic endoscopes (EG 410 HR or EG 205 WR 5; Fujinon) were used. Pharyngeal anesthesia with lidocaine and sedation with midazolam were applied. The precise margins of the lesions were established by CE spraying of 0.1-0.5% indigo carmine solution. At least two samples were obtained from each lesion by ordinary forceps biopsy (Maxum; Wilson-Cook). The biopsy samples were fixed in 8% formaldehyde solution and embedded in paraffin. Endoscopic ultrasonography (GF-UM-130; Olympus) was performed in all cancer cases, if the lesion was > 1 cm in diameter and if the localization of the gastric polyp was suitable for this technique.

2.3.1. Endoscopic mucosal resection procedure

EMR was carried out by the free hand (in 46 cases) or the cap-fitted (in 10 cases) methods (32, 33). After CE, adequate marking surrounding the lesion was performed, using high-frequency electrocautery (PSD 10; Olympus). 2-10 ml of normal saline solution was injected into the submucosal layer beneath the lesion to raise it (NM 200U; Olympus).

The lesion was resected by a snare (SP-5U-1 or SD-17L; Olympus) with high-frequency current (Figs. 2-11). The resected specimens were washed in normal saline, marked, oriented and fixed in 8% formaldehyde solution and embedded in paraffin. Tissues were sliced into 2-

mm sections parallel to the largest diameter of the lesion, and were subjected to hematoxylin and eosin staining and histological study by light microscopy. Slides were coded and examined blindly by the pathologist according to the WHO classification (49), and the cases were later reclassified according to the modified Vienna classification (50, 51). After EMR, the patients participated in sucralfate and proton pump inhibitor therapy. Aspirin treatment, discontinued 7 days before the EMR, was reintroduced 7 days after the procedure. None of the patients received warfarin. All complications occurring during EMR or in the subsequent observation period were documented.

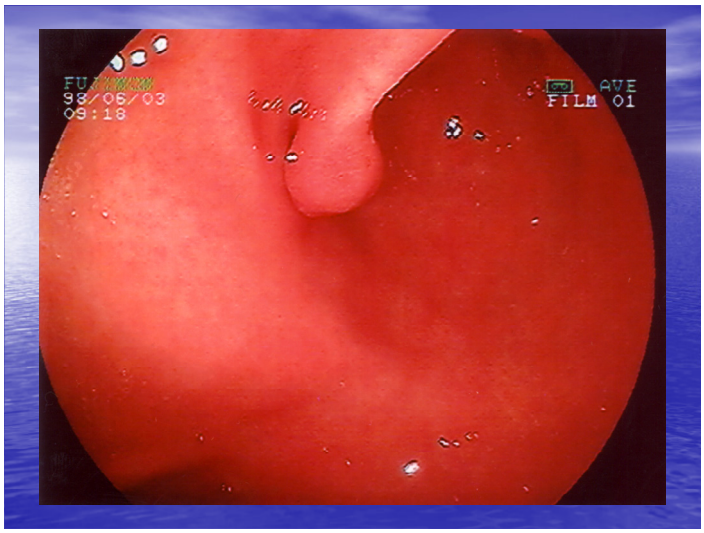

Fig. 2. Small gastric adenoma at the gastric angle

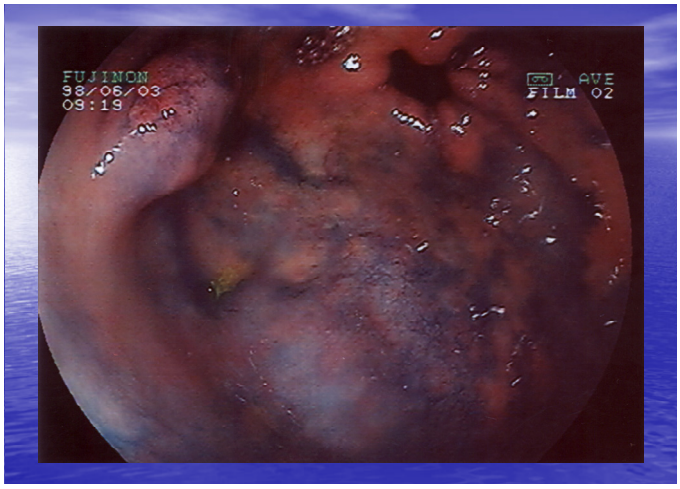

Fig. 3. The same lesion as in Fig. 2. after indigo carmine CE

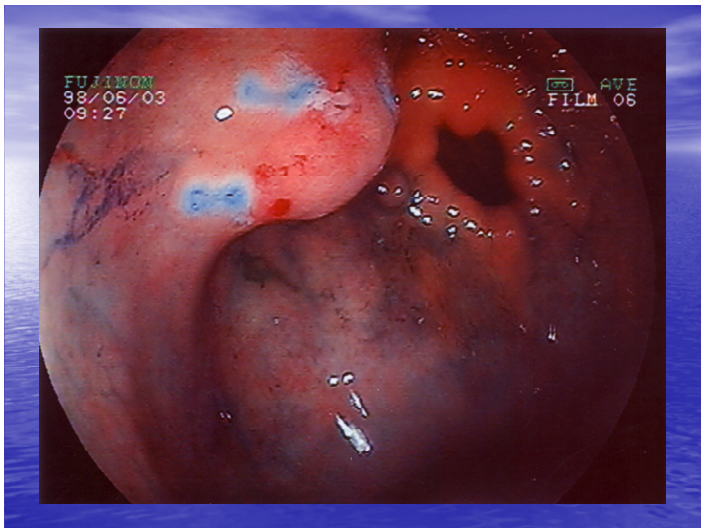

Fig. 4. The lesion after marking before EMR

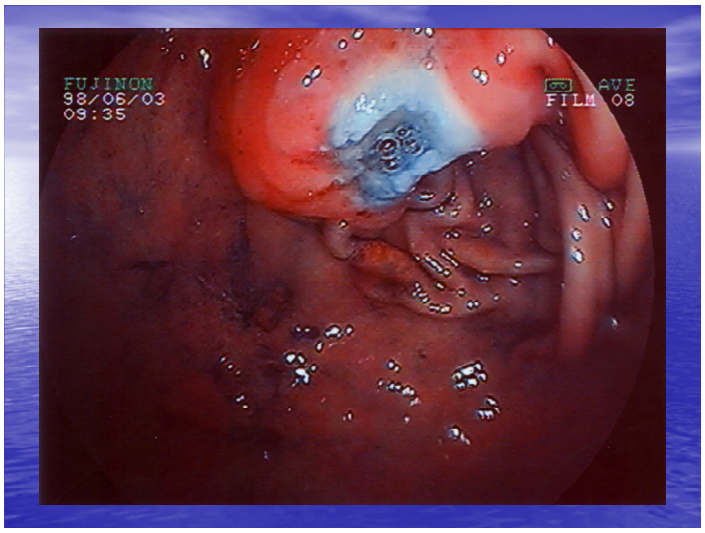

Fig. 5. Arteficial ulcer immediately after EMR of lesion showed at Fig. 4.

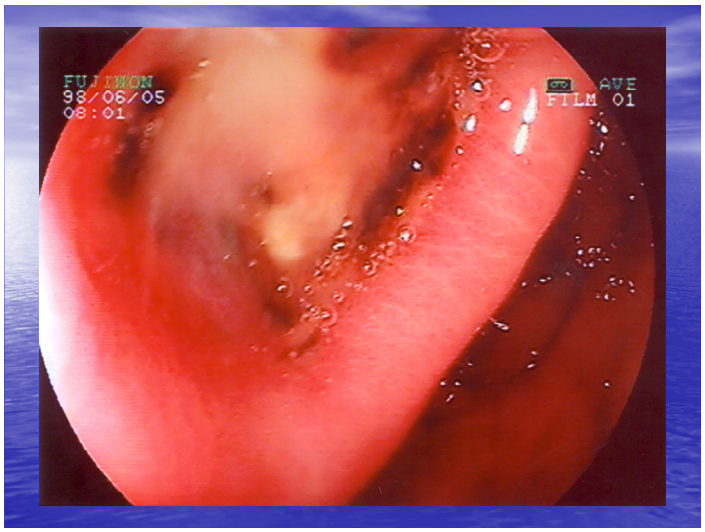

Fig. 6. EMR ulcer after 2 days of procedure

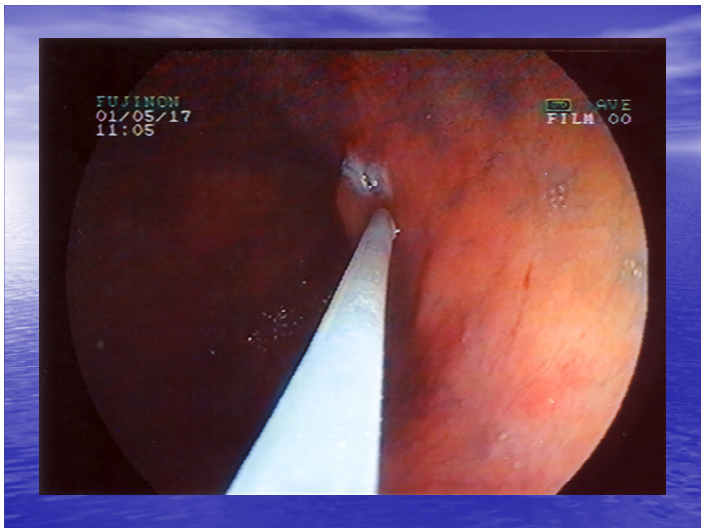

Fig. 7. IIa early gastric cancer in the gastric body, marking the lesion

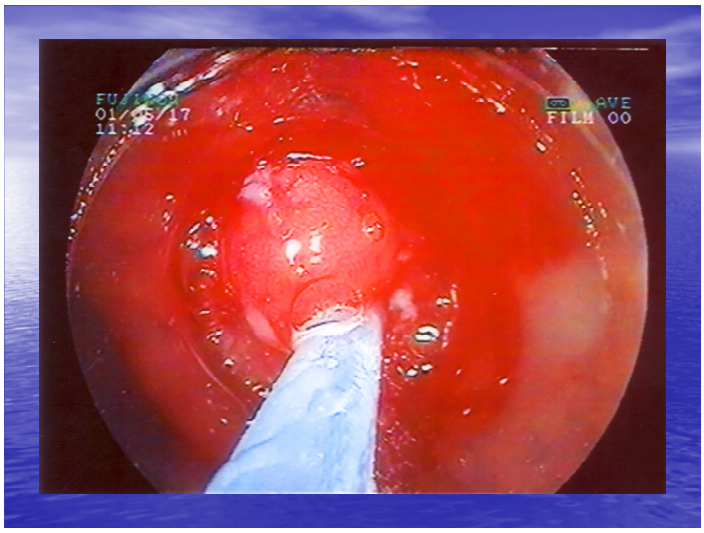

Fig. 8. Snaring with cap-fitted endoscope the same cancer as seen on Fig. 7.

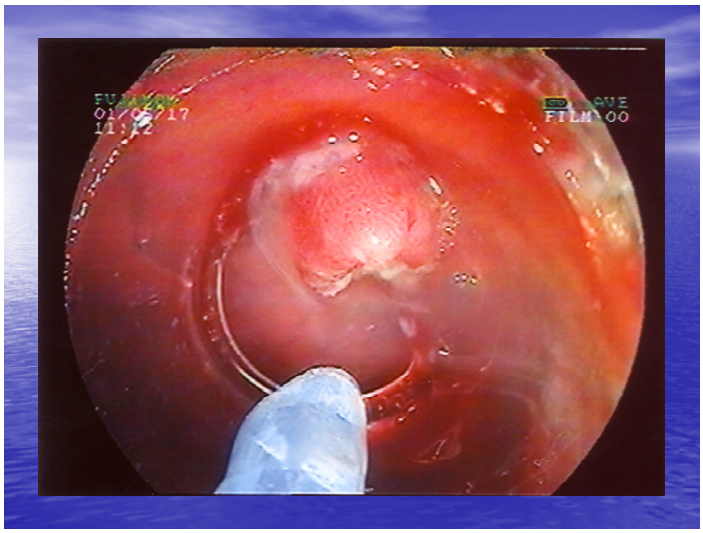

Fig. 9. The entire lesion was resected by cap-fitted endoscope

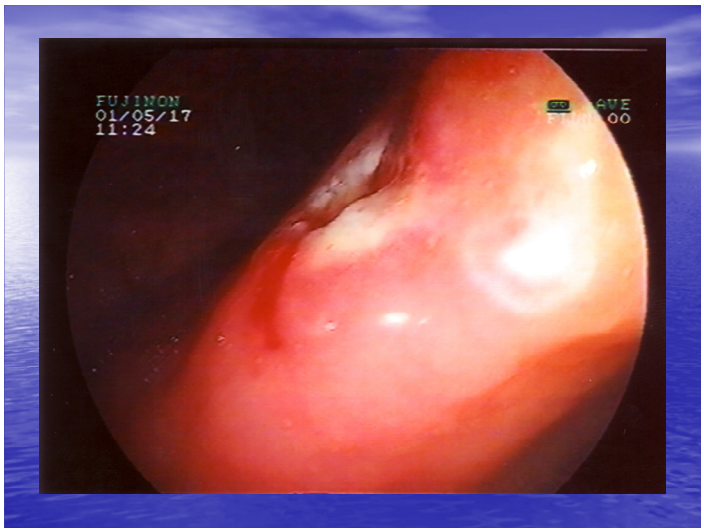

Fig. 10. The EMR ulcer after resection of lesion

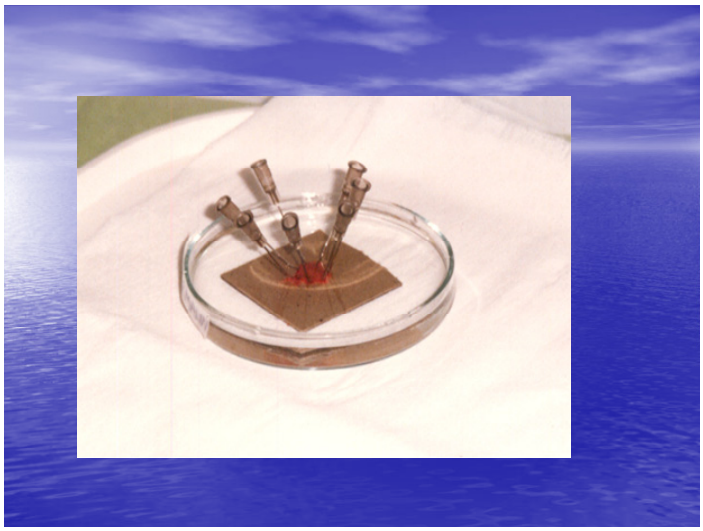

Fig. 11. The resecatum after EMR procedure

3. Results

The contrast staining with indigo carmine highlighted and delineated irregularities in the mucosal architecture. The results could be evaluated immediately after application of the dye. CE facilitated the detection of mucosal changes in esophageal Crohn's disease (52). Contrast CE relies on standard endoscopic observation to detect subtle changes that suggest the presence of a small, flat adenoma or cancer with a slight elevated redness, disruption of the normal vascular network and an altered mucosal groove pattern. When any of these changes are evident, the spraying of contrast-enhancing indigo carmine dye with or without magnification will make the detailed structure of the presumed lesion more evident.

3.1. Chromoendoscopy in the stomach

From 1993 to 1996, gastric adenomas < 1 cm in diameter were found in 5 patients (0.14%) (4 females and 1 male, median age: 69.2 years (58-76 years); and all were removed *in toto* by EMR. The indications were epigastric pain and/or abdominal discomfort. During this period, 3680 patients were investigated with upper GI endoscopes and gastric adenomas > 1 cm in diameter were found in 3 patients (0.22%).

From 1997 to 2000, upper GI endoscopy was performed in 3782 patients. Among them, gastric adenomas < 1 cm in diameter were diagnosed in 14 cases(0.37%) (11 females and 3 males, median age: 68 year /42-78/); all were resected *in toto* by EMR . During this period, 4 patients were found with gastric adenomas with diameters > 1 cm (0.5%).

Indigo carmine contrast staining did not interfere with the rapid urease test or the histological proof of *Helicobacter pylori.*

3.2. Chromoendoscopy in the colon

From 1998 to 1999, 544 patients underwent coloscopy. 87 polyps < 1 cm in diameter were found. 64 were diagnosed as adenomatous after indigo carmine contrast staining via the signs of groove or sulcus mucosal pit pattern (Fig. 12). Finally, 59 were proved to be adenomas by histological assesment and 5 were hyperplastic. The diagnostic accuracy was 92% (53). In my opinion, 23 polyps showed the signs of a hyperplastic (dotted) pattern during endoscopy after the use of indigo carmine. After histological examinations, the diagnostic accuracy was 78% in hyperplastic polyps. In 5 cases, adenomas were diagnosed by the pathologists. The hyperplastic group consisted of hyperplastic polyps, lymphoid aggregates and inflammatory polyps (Table 2). The pattern of adenomas with severe dysplasia was also typical, but this was not enough to draw conclusions. In the colon, the small flat colorectal neoplastic lesions, *i.e.* flat adenomas, may be as translucent as the surrounding mucosa or resemble a small erythema, and could easily be overlooked with conventional colonoscopy. A central depression in flat neoplastic lesions is considered an endoscopic marker for severe dysplasia or carcinoma or a sign of deep invasion.

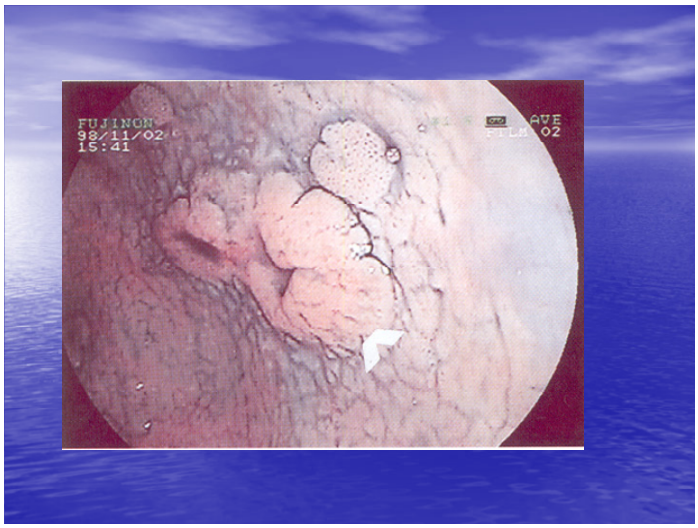

Fig. 12. Small flat lesions in the sigmoid colon. The white arrow showes an adenoma with severe dysplasia proved by biopsy. Above it a biopsy proven hyperplastic polyp with dotted pit pattern.

3.3. Chromoendoscopy in the esophagus

Indigo carmine staining was used in the diagnosis of rare, unusual esophageal manifestations of Crohn's disease. An 18-year-old Caucasian woman presented dysphagia, odynophagia and a 2 kg weight loss. She had oral aphthae and a small perianal fistula. Upper GI endoscopy revealed punchhole-pattern ulcers in the esophagus (Figs. 13 and 14). The stomach and duodenum were normal. The ulcers differed from those seen in herpes simplex virus or cytomegalovirus esophagitis and the known spectrum of esophageal Crohn's disease (52). In this case, indigo carmine contrast staining was an aid to the diagnosis of the punchhole ulcers in the esophagus, and the gastric and duodenal mucosa was demonstrated to display a normal pattern.

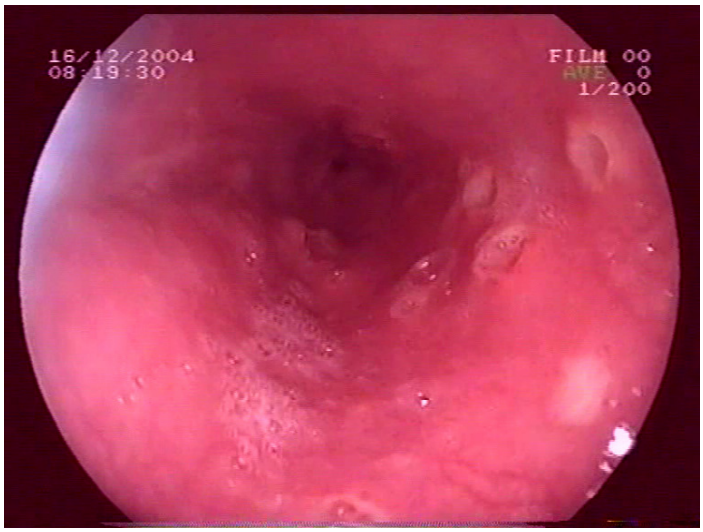

Fig. 13. Esophageal manifestation of Crohn's disease.

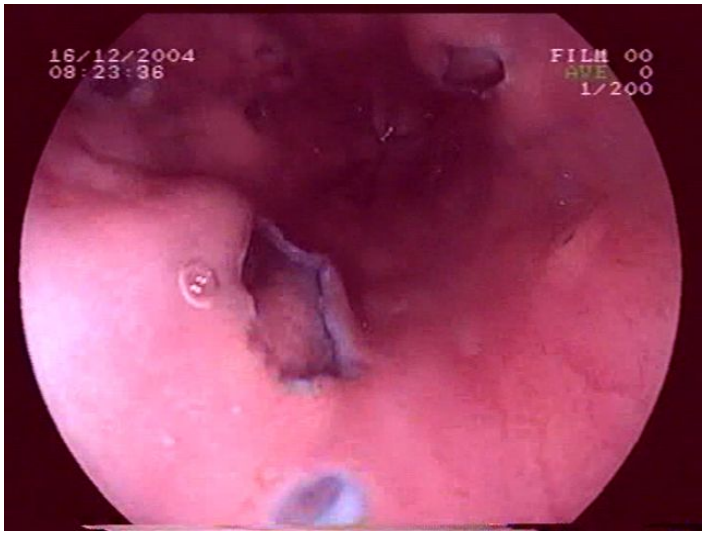

Fig. 14. Esophageal manifestation of Crohn's disease after indigo carmine staining

3.4. Endoscopic mucosal resection

3.4.1. Endoscopic mucosal resection of flat lesions

56 gastric polyps in 44 patients met the inclusion criteria. There were 30 females and 14 males, with a mean age of 67 years (32-83). Two patients had 3 polyps, 8 patients had 2 polyps and the remaining 34 patients had a single polyp. The anatomic locations of the 56 gastric polyps were as follows: 7 in the cardia, 2 in the fornix, 12 in the corpus and 35 in the antrum. The diameters of the polyps lay in the range 0.5-3 cm. The morphology of the lesions was type I in 20, type IIa in 35, and type IIa-IIc in 1 case. All the lesions were flat or sessile polyps, and simple polypectomy was therefore not possible (Table 3).

The histological examination of the forceps biopsy specimens revealed neoplastic lesions in 36 cases and hyperplastic-inflammatory lesions in 18 cases (Table 4). We decided on a second mucosectomy during the EMR procedure in 2 cases because of "suspicious lesions", which is why there are no previous biopsy results in 2 EMR cases.

The histology of the forceps biopsy revealed *in situ* carcinoma in 3, adenoma with no dysplasia in 18, adenoma with low-grade dysplasia in 2, adenoma with moderate-grade dysplasia in 6, and adenoma with high-grade dysplasia in 7 cases.

All neoplastic lesions were diagnosed as being within the mucosal layer by endoscopic ultrasonography. EMR was completed *en bloc* in 54 cases and in two pieces in 2 cases; both were hyperplastic polyps.

Histology of the resected specimen revealed neoplastic lesions in 34 cases and hyperplastic-inflammatory lesions in 21 cases. Adenoma without dysplasia was diagnosed in 14 cases, adenoma with mild dysplasia in 3, with moderate dysplasia in 9, and with severe dysplasia in 1 case (Table 4). In 1 case (the previous biopsy indicated adenoma without dysplasia), the histology of the resected specimens was not informative due to thermal injury. Complete agreement between the histological results on the forceps biopsy sample and the ectomized polyp was seen in 31 (55.3%) polyps. However, there were important disagreements between the histological results on the forceps biopsy specimens and the resected specimens in 12 cases (Tables 5 and 6).

Among the 4 polyps classified as hyperplastic on forceps biopsy 3 were adenomas, 2 of them with mild dysplasia, and 1 was a gastrointestinal stromal tumor (GIST) on EMR; 2 polyps diagnosed as adenomas on forceps biopsy proved to be hyperplastic on EMR. The previous forceps biopsy showed adenomas with severe dysplasia in 4 patients, while the EMR histology revealed *in situ* carcinoma in all of them. *In situ* carcinomas were diagnosed in 3 patients on the basis of the histology of the forceps biopsy, but EMR revealed only 1 cancer: the second was carcinoid and the third was a hyperplastic polyp at the carefull final histologic examination.

3.4.2. Endoscopic mucosal resection of early gastric cancer

7 EGCs were diagnosed via the histological analysis of the resected specimens during this period: 4 females and 3 males, with a mean age at endoscopy of 68 (42-80) years. All 7 patients had severe concomitant diseases.

Only 2 of the 7 EGCs had been diagnosed previously by forceps biopsy. This means that complete agreement between the histological results on the forceps biopsy sample and on the ectomized polyp was seen in only 28.5% of the cases. There was a significant disagreement between the histological results on the forceps biopsy specimens and on the resected specimens in the remaining cases. In 4 EGC cases, the histological result on the previous forceps biopsy was adenoma with high-grade dysplasia in 3 cases, and hyperplastic polyp in 1 case (Table 7). After careful histological study of the resected specimen, the latter case proved to be one of gastrointestinal stromal tumor (GIST), which was confirmed by c-kit (CD117) positivity by using immunohistochemistry. The previous forceps biopsy had shown adenomas with high-grade dysplasia in 3 patients, while the EMR histology demonstrated well-differentiated intramucosal carcinoma in all of them. The previous forceps biopsy had indicated well-differentiated intramucosal carcinoma in 1 case, while the EMR histology revealed well-differentiated neuroendocrine tumor (carcinoid) with chromogranin A immune staining (Fig. 15). Altogether, the sensitivity and specificity of the forceps biopsy procedure for diagnosing neoplastic lesions were 87.5% (95%CI=76.0-98.9%) and 65.2% (95%CI=45.7-84.7), respectively (54).

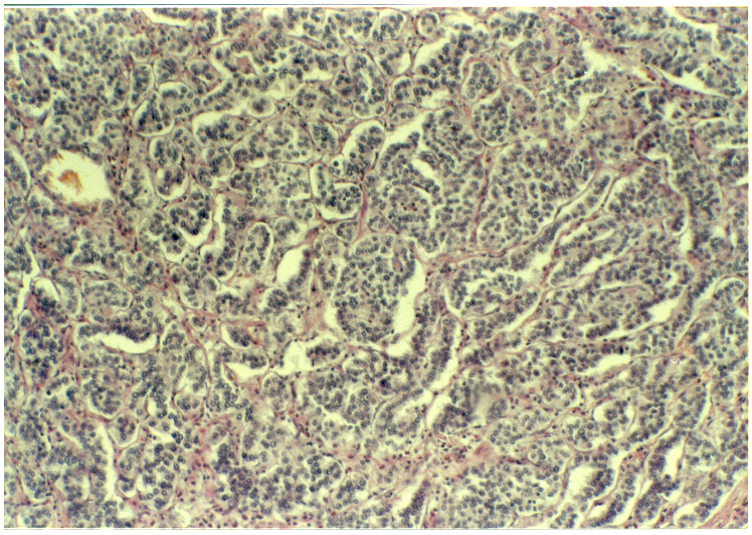

Fig. 15. well-differentiated neuroendocrine tumor (carcinoid) with chromogranin A immune staining

The EMR was considered to be complete in 5 patients. In 2 cases with well-differentiated intramucosal carcinoma, neoplastic glands were detected at the resection lines. In one of these cases, Nd YAG laser therapy was applied. In the other case, the patient

underwent Billroth II resection, but cancer cells were not revealed in the resected specimen. This was probably due to the “burning effect”: the cancer cells at the resection margin were destroyed by the burning effect of the high-frequency current. After the EMR, 6 patients are alive; 1 patient died from a myocardial infarction. Up to her death, the findings of the follow-up endoscopies were normal.

3.4.3. Complications of endoscopic mucosal resection

The complications of EMR include pain, perforation, bleeding and rarely aspiration pneumonia. The pain after EMR is usually mild (55). The mean duration of hospital stay after EMR was 7.6 days; there was no mortality. No serious complications, such as perforation or massive bleeding necessitating surgical treatment, were encountered. Postmucosectomy bleeding was observed in 3 of the 56 cases (5.3%); all were successfully treated by endoscopic hemostasis. One patient required transfusion. Surgical intervention was not needed.

There is no consensus as to how to treat iatrogenic ulcers caused by EMR. Lee *et al*. (56) suggested omeprazol treatment for one week, others sucralfate, and a Japanese endoscopist previously a type 2 histamine receptor antagonist. In our opinion, on the day of EMR, the patient should take oral sucralfate and an oral proton pump inhibitor. If we observe significant bleeding during EMR, proton pump inhibitor infusion is necessary on the day of EMR. If there are anamnestic data of an ulcer, we use a double dose of the inhibitor. In Okano’s retrospective study of 477 EMR cases, the only significant difference between bleeders and non-bleeders after EMR was the presence of bleeding during mucosectomy (57). During EMR or ESD, immediate bleeding can be successfully treated by grasping and coagulation of the bleeding vessels with hot biopsy forceps, or by argon plasma coagulation. Endoclips are also devices often used for this. Delayed bleeding is not common, and may be treated by urgent endoscopy using these techniques. Perforation is uncommon during EMR, but the risk during ESD is about 4%; closure is typically performed with clips (58).

3.4.4. Long-term follow-up after endoscopic mucosal resection

After EMR, our patients were followed up by endoscopy at 3, 6 and 12 months for the first year, and once a year thereafter. As regards all cases up to December 31, 2004, the mean follow- up was 33 months (1-90).

During the long-term follow-up, 3 adenoma and 3 hyperplastic polyp recurrences were observed in 4 patients; in 2 of them, piecemeal EMR was performed. There was 1 recurrence in each of 2 patients and 2 recurrences in each of 2 patients. However, the histology of these recurrent polyps was not identical with the initial histology. In 1 patient, a hyperplastic polyp was detected at the same location1 year after the reappearance and resection of an adenoma. In another patient, 1 year after the resection of a recurrent hyperplastic polyp, an adenoma was revealed at the identical site. 1 metachronous adenoma was detected during the follow-up (59).

In the ECG cases up to December 31, 2006, the mean follow-up time was 38 months (6-72). EGC did not recur during the follow-up.

4. Discussion

4.1. Chromoendoscopy

One of the dreams of GI endoscopists, idealistic though it may be, is to make a diagnosis without taking biopsies. In the field of GI endoscopy, 24 years has passed since the development of the video-endoscope (electronic endoscope) in 1983 as a replacement for the fiber endoscope. Following the advent of CE and high-resolution electronic endoscopy, the discrepancy between the endoscopic and pathological diagnoses can be narrowed. In my thesis, I have tried to provide a short answer concering this problem.

At a concentration of 0.1-0.5%, an aqueous solution of indigo carmine has a deep-blue color that contrasts sharply with the red GI mucosa. Because of gravity, the solution pools in areas of depressions and ulcerations and fills the crevices and valleys between mucosal projections. Thus, contrast staining with indigo carmine highlights and delineates irregularities in the mucosal architecture. The results can be evaluated immediately after application of the dye. Contrast CE relies on standard endoscopic observations to detect subtle changes that suggest the presence of a small, flat adenoma or cancer with a slight elevated redness, disruption of the normal vascular network and an altered mucosal groove pattern. When any of these changes are observed, the spraying of contrast-enhancing indigo carmine dye, with or without magnification, will make the detailed structure of the presumed lesion more evident. The enlargement of the endoscopic picture from 30 up to 50 times, together with contrast staining, produces a virtually microscopic picture of the mucosa. The dye produces a three-dimensional, stereoscopic picture, as can be seen with a 2 charge-

coupled device endoscope (a new type of endoscope). This three-dimensional, stereoscopic picture improves the possibilities in the field of endoscopic diagnosis. Indigo carmine is a well-known, readily available food colorant. Monographs on gastroenterology have suggested its use during both upper and lower endoscopic examinations (60).

Indigo carmine in 0.1-0.5% aqueous solution was used in our endoscopic examinations. The number of small gastric adenoma cases diagnosed increased by threefold. Indigo carmine contrast staning was very useful before EMR to delineate the lesions. The histologic types of small flat colonic lesions can be predicted. Of course, histological examination of a removed polyp remains the gold standard, but in cases of endoscopically not removable small lesions, or in the ”missed polyp case” after polypectomy, it is a useful method for the follow-up and to give an appropriate decision regarding such lesions.

Indigo carmine is also useful for investigating ulcer healing and to differentiate benign or malignant ulcers endoscopically. The abrupt tapering of wrinkles of the mucosa, abrupt ending of the mucosa, a worm-eaten pattern, *etc*. are easily recognized in a malignant ventricular ulcer. The nature of lesions covered by neoplastic mucosa becomes evident by analyzing the surface pattern. The visualization of normal and abnormal vascular patterns, spots with unusual color, reflection differences, bends or bending is easier with the indigo contrast method.

In Japan, the colonic pit patterns are divided into 3, 4, 5 or 6 categories (varying from author to author), but the basic groups are normal, hyperplastic, adenomatous and carcinomatous (61).

Kato *et al*. could achieve a diagnostic accuracy of 75% in non-neoplastic cases, of 94% in adenomatous polyp cases and of 85% in invasive cancer cases on the use of magnifying colonoscopes and indigo carmine contrast staining (62). They applied Kudo’s staging for pit pattern analysis, which in my opinion is difficult, and not widely accepted, even in Japan (63). The method of Mitooka and Axelrad was used instead, which was easily applicable to minute flat colon lesions and did not involve cancer recognition, where in my opinion we should be more critical and accurate and where histological diagnosis is the *sine qua non* (47, 64).

The recognition of flat adenomas is important, because we found them more frequently than we thought earlier, and they play an important role in colon carcinogenesis. For flat adenomas, we could recognize small depression by using indigo carmine contrast staining; this could be a sign of severe dysplasia.

The endoscopic diagnosis of inflammatory bowel diseases is usually easy for typical cases. The use of indigo carmine contrast staining is effective in the detection of early or very

mild cases, the decision concerning the intactness of areas lying between evidently involved segments, and the detection of dysplastic area in longstanding colitis. In one prospective, back-to-back colonoscopy surveillance study involving 100 patients with chronic ulcerative colitis, an indigo carmine-targeted biopsy protocol required fewer biopsies, yet tended toward a significant increase in dysplasia detection as compared with conventional colonoscopy and random biopsy (65). In my opinion, indigo carmine contrast staining is very useful to choose the appropriate loci for taking biopsy specimens.

The diagnosis of esophageal Crohn's disease is often difficult. The spectrum of esophageal Crohn's disease may vary from mild esophagitis with small erosions to transmural involvement with perforation and fistualization to adjacent organs (66). Vertical lines in the esophageal mucosa, aphthous ulcers, deep ulcers, erythematous nodules and polypoid lesions, pseudomembrane formations, strictures and the formation of multiple mucosal bridges have been described (67, 68). As far as I know, ours was the first report of punchhole ulcers as an esophageal manifestation of Crohn's disease. As this case demonstrated, such ulcers may be the first sign of Crohn's disease. Awareness of this possibility may expedite the diagnosis (52). The histology showed a severe inflammatory reaction in all biopsies taken from the esophagus. There was no granulation tissue in the mucosa, but this deep chronic inflammation with plasma cells and lymphocytes extended into the lamina propria, and there were edematous changes in the surface epithelium.

In conclusion, indigo carmine contrast staining can be used in gastroenterology to enhance endoscopic visualization throughout the GI tract. We found that CE gave greatly accentuated abnormalities in the mucosal architecture, and it was possible to discriminate between various pit patterns. To improve minute endoscopic observation, CE is a suitable method.

4.2. Endoscopic mucosal resection

In 3% of upper GI endoscopy examinations, gastric polyps can be seen (69). Adenomatous gastric polyps compromise 5-10% of gastric polyps and are considered to be premalignant. The incidence of malignant transformation is in the range 4-70% (70, 71). The risk varies as concerns size, endoscopic appearance and histological type. The occurrence of hyperplastic gastric polyps is 70-90% of that of gastric polyps. The hyperplastic polyps have malignant foci in 1.5-3% (70-73). A precise histological diagnosis and the reliability of the histological results of forceps biopsy sampling with regard to the entire lesion are therefore essential for the therapeutic decision.

EMR was initially developed in Japan for the resection of EGC and esophageal cancer (32, 33, 74). The main indications for EMR are: curative for early cancers, palliative for those who are not fit for operation, and finally diagnostic indications. It is well known that endoscopic mucosal biopsies obtained with standard biopsy forceps can give false-negative results, especially if the epithelial layer is not involved in the pathological process. EMR offers *en bloc* resection of the entire lesion. The histological examination of the whole lesion can be more reliable than forceps biopsy, which may not be representative of the entire lesion. However, EMR was not frequently used for this purpose in eastern countries (17).

In this prospective bicenter study, complete agreement between the histological results of the forceps biopsy and the resected specimens was accomplished in 55.3% of the flat lesions. A clinically relevant discrimination between neoplastic and hyperplastic lesions was not achieved in 7 polyps. In 14 neoplastic and 1 hyperplastic polyps, the degree of dysplasia seen at the histological examinations of the forceps biopsies and the resected specimens differed. In 4 cases, foci of carcinoma were present in the resected specimens, but were missed by the biopsy sampling. In 1 case, the histological types of the neoplastic cells in the biopsy samples and the resected specimen were basically different (epithelial carcinoma – carcinoid). A flat lesion classified as hyperplastic on forceps biopsy proved to be a GIST on EMR. A polyp was diagnosed as containing foci of carcinoma on forceps biopsy, while the histology of the resected specimen revealed a hyperplastic polyp. In this case, the regenerative alterations in the erosive area of the polyp were overestimated as intraepithelial neoplasia. These disagreements considerably alter the management of our patients (54, 59).

The diagnostic efficacy of histology on a forceps biopsy sample was earlier compared with that of examination of the entire polyp. In a study of 24 ventricular polyps, Seifert *et al*. (75) demonstrated that the histological diagnosis on the polypectomy specimen did not coincide with the results on the preliminary biopsy samples in 75% of the cases. Ginsberg *et al*. (14) later showed that histology on forceps biopsy samples may be faulty. In their prospective trial on 222 gastric polyps, Muehldorfer *et al*. (15) found complete agreement between the histological results on the biopsy and polypectomy specimens in only 55.8% of the cases. It might be believed that a forceps biopsy specimen is more representative of an entire flat polyp than that of a pedunculated one, since its volume is less and the surface from which the biopsy is taken is larger. However, our results on flat gastric lesions (complete agreement in only 55.3% of the cases) compare well with those observed by Muehldorfer *et al.*

The degree of dysplasia found in the adenomatous polyps differed between the forceps biopsy samples and the ectomized specimens (76). Both under- and overestimation occurred in the diagnosis on the biopsy samples. This is not highly relevant clinically as all adenomas must be removed completely, because of the adenoma-carcinoma sequence. Parks *et al*. (77) emphasized that grading the dysplasia from a forceps biopsy sample is useless, because mild dysplasia can not rule out the focus of malignancy in some other part of the adenoma. On the other hand, in contradiction with previous belief, there may be carcinomatous foci even in a hyperplastic polyp 5 mm in diameter (70, 73). In our series, there was no hyperplastic polyp which contained malignant foci. However, some flat lesions would have been misdiagnosed according to the histology on the forceps biopsy sample (34, 54). The biopsy specimens do not represent the whole lesion: the material may be insufficient to establish the correct diagnosis, or focal cancers may be missed. It is therefore recommended to remove flat lesions at the time of endoscopy in order to establish the final diagnosis after the thorough histological examination of the entire lesion. As the majority of gastric polyps are benign, EMR in most cases is curative (71).

We observed 6 (10.7%) recurrences during the follow up period. This recurrence rate is higher than those (6.1 and 5.9%) observed by other investigators (78, 79). The discrepancy may be explained by our longer follow-up period (33 months) and the incomplete resection due to our inexperience at the beginning of the study. None of the EGC cases recurred during the long-term follow-up. Two recurrences were diagnosed in 2 patients. However, the histology of the recurrent polyps was not identical with that of the previously ectomized polyps. This apparent contradiction could be explained in that hyperplastic polyps may contain adenomatous foci, and adenomatous polyps may carry hyperplastic areas (14) that were not resected during EMR.

The recommendation to remove all flat lesions completely by means of EMR must be weighed against the possible complications of this procedure. In our study, bleeding occurred in 3 patients (5.3%), all of whom were treated successfully endoscopically. One patient required transfusion. Surgical intervention was not needed. In this series of EMR, we reported a case in which aspiration pneumonia occurred after a long-lasting, difficult EMR (59).

In view of our acceptable complication rate and the mildness of the complications, complete endoscopic removal of all flat lesions > 5 mm should be considered. There is no consensus as to how to treat iatrogenic ulcers caused by EMR. Lee *et al*. (56) suggested omeprazol treatment for 1 week, while others proposed sucralfate administration and a Japanese endoscopist recommended a histamine-2 receptor antagonist. In our opinion, on the

day of EMR the patient should take oral sucralfate and an oral proton pump inhibitor. If significant bleeding is observed during EMR, infusion of such an inhibitor is necessary on the day of EMR. If there is a history of ulcer, we use a double dose of inhibitor. In Okano's retrospective study of 477 EMR cases, the only significant difference between bleeders and non-bleeders after EMR was the occurrence of bleeding during mucosectomy (57). EMR provides diagnostic and staging advantages in the assessment of patients with EGC as compared with forceps biopsy, because it furnishes more intact mucosa and submucosa for histological analysis (54).

When we started our study, we recognized the result of Nakijama (35). Later, there were trials to expand the indication of EMR, as a local treatment. The presence of lymph node metastasis is the most important prognostic factor for patients with EGC.

The results of our study demonstrate that a specimen taken by forceps biopsy is not fully representative of the entire flat polyp. It may lead to a faulty differentiation between neoplastic and non-neoplastic lesions. Dysplasia in a forceps biopsy sample does not exclude the presence of foci of carcinoma in some other part of the adenoma, and hyperplastic lesions may contain adenomatous foci. Accordingly, it is recommended to remove all flat lesions > 5 mm *in toto* endoscopically. This ensures a precise final diagnosis and possibly definitive treatment. EMR is a safe and not excessively expensive diagnostic, curative and palliative method, and we suggest its wider use in Europe (80). As gastroenterologists in Hungary become more familiar and experienced with techniques of EMR and ESD (the latter was not a topic of this thesis), patients will benefit from these minimally invasive therapeutic techniques.

4.3. The future

CE is not standardized for several stains and the staining patterns are subject to observer interpretation. There is a need to build a consensus on staining techniques and terminology of the mucosal patterns for most applications, in addition to proving efficacy and reproducibility in high-quality, randomized, controlled trials before CE can be incorporated into routine clinical practice. The cost-effectiveness of tissue staining for various GI conditions has not been established, and its stance relative to commercially available, competing, and less cumbersome "CE without dye" techniques (see below) remains to be seen (81).

There are some new methods as competitors for CE.

FICE

FICE (Fuji Intelligent Color Enhancement) is a software application that can be switched on by pressing a button on the endoscope; the best image can be reconstructed by choosing various wavelengths, depending on the depth and spectral reflectance of the targeted tissue. It is possible to select a large number of wavelength combinations for a differentiated display of the mucosa. Thus, latent mucosal lesions can be diagnosed without intravital staining (82).

Confocal endomicroscopy and endocytoscopy

Confocal endomicroscopy (Pentax) appears to be particularly promising for the diagnosis of EGC in flat lesions. It is a patented technological advance that provides *in vivo* subsurface optical histological information with a high degree of sensitivity in real time during ongoing endoscopy. The adjunctive procedure will not replace pathological tissue diagnosis, but it is likely to have a significant impact on endoscopic diagnostic and therapeutic algorithms, potentially making it possible to take targeted biopsies in the future, for example, or facilitating rapid diagnostic decision-making for EMR (83). The real significance of this technique remains to be investigated, but it appears that the potential future impact of confocal endomicroscopy will extend far beyond that of morphological imaging alone (84).

In vivo endocytoscopy (Olympus XEC 300 and 120) is a newly developed tool that permits microscopic imaging of living cells from the GI mucosa during routine endoscopy. The newly discovered diagnostic possibility of endocytoscopy may be of significant importance in clinical practice and lead to a rapid diagnosis of neoplastic changes during ongoing endoscopy (85).

NBI

NBI (narrow-band imaging) system is based on modification of the spectral features by narrowing the bandwidth of spectral transmittance with optical filters. It provides a unique image emphasizing the capillary pattern and the surface structure. The NBI system is sufficient to differentiate non-neoplastic lesions from neoplastic lesions (optical CE); it includes a special feature allowing otherwise invisible endoscopic findings to be visualized

without a dye solution (high-contrast endoscopy) (86). Tischendorf *et al*. recently reported a comparative study between magnification using either CE or narrow band imaging of 200 colorectal polyps. NBI was equal in specificity, but superior in sensitivity to differentiate between neoplastic and non-neoplastic polyps (87). Taken overall, the results suggest that, although NBI may enhance the detection of various neoplastic and preneoplastic lesions, further research is needed before this method can be recommended for routine use in these settings (88).

4.4. Endoscopic submucosal dissection (ESD)

ESD is a relatively new technique that has been used to resect larger (> 20 mm) mucosal lesions in the stomach. After careful examination the target lesion is first marked, with coagulation current from the tip of a knife approximately 5 mm around the margins of the tumor. The entire marked area is then elevated with a submucosal injection. A needle knife is next used to cut along the outside of the margins, while the lesion is still elevated. Dissection along the submucosal plane is then performed using tools such as a hook knife (89) or flex knife (90) or the knife in a small cap technique (91). For ESD, Yamamoto (92) described a reliable method for the *en bloc* resection of large superficial lesions. Locally injected sodium hyaluronate (and/or glycerol and/or hypertonic sugar solution) (93) produced a long-lasting mucosal protrusion, and a small caliber tip transparent hood was applied to open the incised mucosa for better visualization of the submucosal tissues. He emphasized the effective control of bleeding during the procedure (with argon plasma coagulation and/or hemostatic forceps) as a key element for success. Perforation, a major concern as a possible complication of ESD, can also be prevented by sufficiently thickening the mucosa by appropriate injection of sodium hyaluronate and careful selection of the incising layer.

This technique takes longer to perform than traditional EMR techniques, and requires that the endoscopist should be experienced with these various tools; larger studies are needed to assess the reliability (93, 94). ESD is superior to standard EMR and has the advantage of achieving large *en bloc* resections; it allows precise histological staging and may prevent disease recurrence as compared with standard EMR methods (95). ESD techniques are rapidly gaining popularity in Japan.

Kume *et al*. recently developed and patented a new type of dissection knife, the irrigation cap-knife, which uses a fixed snare that facilitates ESD by just sliding over the muscle layer with a coagulating current (96).

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6. References

1. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
2. Parkin DM, Whelan SL, Ferlay J: Cancer incidence in five continents. Lyon International Agency for Research on Cancer Press (IARC publication no. 143); *Oxford University Press,* 1997.
3. Hundahl SA, Philips JL, Menck HR: The National Cancer data base report on poor survival in U.S. gastric carcinoma patients treated with gastrectomy. *Cancer* 2000; **88**: 921-927.
4. Noguchi Y, Yoshikawa T, Tsuburaya A, Motohashi H, Karpeh MS, Brennan MF: Is gastric carcinoma different between Japan and the United States? A comparison of patient survival among three institutions. *Cancer* 2000; **89**: 2237-2240.
5. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 2nd English edition. *Gastric Cancer* 1998; **1**: 10-24.
6. Sasako M, Kinoshita T, Maruyama K: Prognosis of early gastric cancer. *Stomach Intestine* 1993; **28**: 139-146.
7. Soetikno R, Kaltenbach T, Yeh R, Gotoda T: Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. *J Clin Oncol.* 2005; **23**: 4490-4498.
8. Giovannini M, Bernardini D, Moutardier V, Monges G, Houvenaeghel G, Seitz JF, Derlpero JR : Endoscopic mucosal resection: results and prognostic factors in 21 patients. *Endoscopy* 1999; **31**: 698-701.
9. Wehrmann T, Lange M, Nakamura A, Riphaus A, Stergiou N: Endoskopische Mukosaresektion von [pra] malignen Veranderungen im oberen Gastrointestinaltrakt. *Z Gastroenterol* 2001; **39**: 919- 928.
10. Simon L, Domján L, Fehér I: A korai gyomorrák endoszkópos rezekciója. *Lege Artis Medicinae* 1992; **2**: 842-847. [Hungarian]
11. Szalóki T: A korai gyomorrák néhány problémája. Új lehetőség a gastroenterologiában: endoscopos mucosa resectio. In. *A daganetellenes kűzdelem Pest Megyében* (szerk: Iglói F.) Budapest, 9-13, 1995.
12. Everett SM, Axon AT: Early gastric cancer in Europe. *Gut* 1997; **41**: 142-150.
13. Remmele W, Kolb EF: Malignant transformation of hyperplasiogenic polyps of the stomach – case report. *Endoscopy* 1978; **10:** 63-65.

14. Ginsberg GG, Al-Kawas FH, Fleischer DF, Reilly HF, Benjamin SB: Gastric polyps: Relationship of size and histology to cancer risk. *Am J Gastroenterol* 1996; **91**: 714-717.

15. Muehldorfer SM, Stolte M, Martus P, Hahn EG, Ell C: Diagnostic accuracy of forceps biopsy versus polypectomy for gastric polyps: a prospective multicentre study. *Gut* 2002; **50**: 465-470.

16. Gotoda T: Endoscopic resection of early gastric cancer: the Japanese perspective. *Curr opin Gastroenterol* 2006; **22**:561-569.

17. Gotoda T, Friedland S, Hamanaka H, et al: A learning curve for advanced endoscopic resection. *Gastrointest Endosc* 2005; **62**: 866-867.

18. Ida K, Kawai K, Koori Y, et al: Application of dye method to gastroscopy. *Gastroenterol Endosc* 1973; **15**: 37-46.

19. Canto MI: Staining in gastrointestinal endoscopy: the basics. *Endoscopy* 1999; **31**: 479-486.

20. Acosta MM, Boyce HWJ: Chromoendoscopy – where is it useful? *J Clin Gastroenterol* 1998; **17**: 13-20.

21. Technology Assesment Committee. Endoscopic tissue staining and tattooing. *Gastrointest Endosc* 1996; **43**: 652-657.

22. Tada M, Kawai K: Research with the endoscope: new techniques using magnification and chromoscopy. *Clin Gastroenterol* 1986; **15**: 417-437.

23. Rácz I, Tóth E: Chromoendoscopic study of the gastric mucosa. *Orv Hetil* 1989; **49**: 2635-2638.

24. Tóth E, Sjölund K, Fork FT, Lindström C: Chronic atrophic fundic gastritis diagnosed by a modified Congo red test. *Endoscopy* 1995; **27**: 654-658.

25. Siegel LM, Stevens PD, Lightdale CJ, et al: Combined magnification endoscopy with chromoendoscopy in the evaluation of patients with suspected malabsorption. *Gastrointest Endosc* 1997; **46**: 226-230.

26. Axelrad LM, Fleischer DE, Geller AJ, et al: High-resolution chromoendoscopy for the diagnosis of diminutive polyps: implications for colon cancer screening. *Gastroenterology* 1996; **110**: 1253-1258.

27. Kudo S, Kashida H, Nakajima T, Tamura S, Nakajo K: Endoscopic diagnosis and treatment of early colorectal cancer. *World J Surg* 1997; **21**: 694-701.

28. Jaramillo E, Watanabe M, Slezak P, Rubio C: Flat neoplastic lesions of colon and rectum detected by high-resolution video endoscopy and chromoscopy. *Gastrointest Endosc* 1995; **42**: 114-122.
29. Matsukuma A, Furusawa M, Tomoda H, Seo Y: A clinicopathological study of asymptomatic gastric cancer. *Br J Cancer*. 1996; **74**: 1647-1650.
30. Soetikno R, Kaltenbach T, Yeh R, Gotoda T: Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. *J Clin Oncol*. 2005; **23**: 4490-4498.
31. Kim, HS, Lee, DK, Baik, SK, Kim, JM, Kwon, SO, Kim, DS, Cho, MY: Endoscopic mucosal resection with ligation device for early gastric cancer and precancerous lesions: comparison of its therapeutic efficacy with surgical resection. *Yonsei Med J* 2000; **41**: 577-583.
32. Tada M, Murakami A, Karita M et al: Endoscopic resection of early gastric cancer. *Endoscopy* 1993; **25**: 445-450.
33. Inoue H, Takeshita K, Hori H et al: Endoscopic mucosal resection with a cap-fitted panendoscope for esophagus, stomach, and colon mucosal lesions. *Gastrointestinal Endoscopy* 1993; **39**: 58-62.
34. Hull MJ, Mino-Kenudson M, Nishioka NS, Ban S, Sepehr A, Puricelli W, Nakatsuka L, Ota S, Shimizu M, Brugge WR, Lauwers GY: Endoscopic mucosal resection: an improved diagnostic procedure for early gastroesophageal epithelial neoplasms. *Am J Surg Pathol* 2006; **30**: 114-118.
35. Nakajima T: Tabular analysis of 10 000 cases of gastric cancer in CIH (in Japanese) *Jpn J Cancer Chemother* 1994; **21**: 1813-97.
36. Yamao T, Shirao K, Ono H, Kondo H, Saito D, Yamaguchi H, Sasako M, Sano T, Ochiai A, Yoshida S: Risk factors for lymph node metastasis from intramucosal gastric carcinoma. *Cancer.* 1996; **77**: 602-606.
37. Gotoda T, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y: Incidence of lymph node metastasis from early gastric cancer: estimation with a lagre number of cases at two large centers. *Gastric Cancer*. 2000; **3**: 219-225.
38. Soetikno R, Kaltenbach T, Yeh R, Gotoda T. Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. *J Clin Oncol* 2005; **23**: 4490-4498.
39. Tada M, Shimada M, Murakami F: Development of strip-off biopsy. *Gastroenterol Endosc* 1984; **26**: 833-839.
40. Martin TR, Onstad GR, Silvis SE, Vennes JA: Lift and cut biopsy technique for submucosal samplings. *Gastrointest Endosc* 1976; **23**: 29-30.

41. Takekoshi T, Baba Y, Ota H, Kato Y, Yanagisawa A, Takagi K, Noguchi Y: Endoscopic resection of early gastric carcinomas: results of a retrospective analysis of 308 cases. *Endoscopy* 1994; **26**: 352-358.

42. Kume K, Yamasaki M, Kubo K, Mitsuoka H, Oto T, Matsuhashi T, Yamasaki T, Yoshikawa I, Otsuki M: EMR of upper GI lesions when using a novel soft, irrigation, prelooped hood. *Gastrointest Endosc* 2004: **60**: 124-128.

43. Akiyama M, Ota M, Nakajima H, Yamagata K, Munakata A: Endoscopic mucosal resection of gastric neoplasms using a ligating device. *Gastrointest Endosc* 1997; **45**: 182-186.

44. Soetikno RM, Gotoda T, Nakanishi Y, Soehendra N: Endoscopic mucosal resection. *Gastrointest Endosc* 2003; **57**: 567-579.

45. Tanabe S, Koizumi W, Mitomi H, Nakai H, Murakami S, Nagaba S, Kida M, Oida M, Saigenji K: Clinical outcome of endoscopic aspiration mucosectomy for early stage gastric cancer. *Gastrointest Endosc* 2002; **56**: 708-713.

46. Czakó L, Szalóki T, Lonovics J: A japán endoszkópos technika. *Magyar Belorvosi Archivum* 2002; **55**: 29-35.

47. Axelrad AM, Fleischer DE, Geller AJ, et al: High resolution chromoendoscopy for the diagnosis of diminutive colon polyps: implications for colon cancer screening. *Gastroenterology* 1996; **110**: 1253-1258.

48. Carr-Locke DL, Benjamin SB, Fleischer DE: Advanced endoscopic techniques. *Advanced Medical Ventures Ltd.* Chicago, CD-ROM 1998.

49. Jass JR, Sobin LH, Watanabe H: The World Health Organization's histologic classification of gastrointestinal tumors. A comentary on the second edition. *Cancer* 1990; **66**: 2162-2167.

50. Sugai T, Habano W, Uesugi N, et al.: Molecular validation of modified Vienna classification of colorectal tumors. *J Mol Diagn* 2002; **4**: 191-200.

51. Schlemper RJ, Riddel RH, Kato Y: The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255.

52. Szalóki T, Tóth V, Czakó L: Unusual esophageal manifestations of Crohn's disease. *Gastrointest Endosc* 2006; **64**: 121.

53. Szalóki T: Indigókarmin festékkontraszt módszer alkalmazása nagy felbontóképességű elektronikus endoszkópia során. *Orv Hetil* 2002; **143**: 25-29.

54. Szalóki T, Tóth V, Németh I, Tiszlavicz L, Lonovics J, Czakó L: Endoscopic mucosal resection: not only therapeutic, but a diagnostic procedure for sessile gastric polyps. *J. Gastroenterol Hepatol* 2007 (in press).

55. Kaneko E, Hanada H, Kasugai T, et al: Survey of gastrointestinal endoscopic complications in Japan. *Gastroenterol Endosc* 2000; **42**: 308-313.

56. Lee SY, Kim JJ, Lee JH, et al: Healing rate of EMR-induced ulcer in relation to the duration of treatment with omeprasol. *Gastrointest Endosc* 2004; **60**, 213-217.

57. Okano A, Hajiro K, Takakuwa H, et al: Predictors of bleeding after endoscopic mucosal resection of gastric tumors. *Gastrointest Endosc* 2003; **57**: 687-690.

58. Minami S, Gotoda T, Ono H, Oda I, Hamanaka H: Complete endoscopic closure using endoclips for gastric perforation during endoscopic resection for early gastric cancer can avoid emergent surgery. *Gastrointest Endosc* 2006; **63**: 596-601.

59. Szalóki T, Tóth V, Tiszlavicz L, Czakó L: Flat gastric polyps: results of forceps biopsy, endoscopic mucosal resection, and long-term follow-up. *Scand J Gastroenterol* 2006; **41**: 1105-1109.

60. Cotton PB, Williams CB: Practical Gastrointestinal Endoscopy, *Blackwell, Oxford*, 1990, p46, p157, p212, p235.

61. Kudo S, Rubio CA, Teixeira CR, et al: Pit pattern in colorectal neoplasia: endoscopic magnifying view. *Endoscopy* 2001; **33**: 367-373.

62. Kato S, Fujii T, Koba I, et al: Assesment of colorectal lesions using magnifying coloscopy and mucosal dye spraying: can significant lesions be distinguished? *Endoscopy* 2001; **33**: 306-310.

63. Kudo A, Hirota S, Nakijima T, et al: Colorectal tumours and pit pattern. *J Clin Pathol* 1994; **47**: 880-885.

64. Mitooka H, Fujimori T, Maeda S, et al: Minute flat depressed neoplastic lesion of the colon detected by contrast chromoscopy using indigo carmine capsule. *Gastrointest Endosc* 1995; **41**: 453-459.

65. Rutter MD, Saunders BP, Schofield G, et al: Pancolonic indigo carmine dye spraying for the detection of dysplasia in ulcerative colitis. *Gut* 2004; **53**: 256-60.

66. Ramaswamy K, Jacobson K, Jevon G, et al: Esophageal Crohn disease in children: a clinical spectrum. *J Pediatr Gastroenterol Nutr* 2003; **36**: 454-458.

67. Rudolph I, Goldstein F, DiMarino AJ Jr: Crohn's disease of the esophagus: Three cases and a literature review. *Can J Gastroenterol* 2001; **15**: 117-122.

68. Decker GA, Loftus EV Jr, Pasha TM, et al: Crohn's disease of the esophagus: clinical features and outcomes. *Inflamm Bowel Dis* 2001; **7**: 113-119.

69. Classen M, Tytgat GNJ, Lightdale CJ, et al: Gastroenterological Endoscopy. *Georg Thieme Verlag, Germany*, 2002, p.509.

70. Orlowska J, Jarosz D, Pachlewski J, Butruk E: Malignant transformation of benign epithelial gastric polyps. *Am J Gastroenterol* 1995; **90**: 2152-2159.

71. Harju E: Gastric polyposis and malignancy. *Br J Surg* 1986; **7**: 532-533.

72. Daibo M, Itabashi M, Hirota T.: Malignant transformation of hyperplastic polyps. *Am J Gastroenterol* 1987; **82**: 1016-1025.

73. Remmele W, Kolb EF: Malignant transformation of hyperplasiogenic polyps of the stomach – case report. *Endoscopy* 1978; **10**: 63-65.

74. Lambert R.: Treatment of esophagogastric tumors. *Endoscopy* 2003; **35**: 118-126.

75. Seifert E, Elster K: Gastric polypectomy. *Am J Gastroenterol* 1975; **63**: 451-456.

76. Szalóki T, Tóth V, Tiszlavicz L, Czakó L: A gyomor endoszkópos mucosaresectiója-diagnosztikus és terápiás módszer. *Orv Hetil* 2006: **147**: 501-507.

77. Park DI, Rhee PL, Kim JE, et al: Risk factors suggesting malignant transformation of gastric adenoma: univariate and multivariate analysis. *Endoscopy* 2001; **33**: 501-506.

78. Cheon YK, Ryu CB, Ko BM, et al: Long-term Follow-up Results of Endoscopic Mucosal Resection for Early Gastric Cancer and Gastric Flat Adenoma. *Korean J Gastrointest Endosc* 2000; **21**: 891-897.

79. Seifert E, Gail K, Weismuller J: Gastric polypectomy. Long-term results (survey of 23 centres in Germany). *Endoscopy* 1983; **15**: 8-11.

80. Szalóki T, Czakó L, Tóth V: Diagnosis and treatment of superficial neoplastic lesions of the stomach by endoscopic mucosal resection. *In. Gastric Cancer Research Trends (ed: Tompkins M.B.) Nova Science Publishers.* New York. 2008 (in press).

81. American Society for Gastrointestinal Endoscopy: Chromoendoscopy. *Gastrointest Endosc* 2007: **66**: 639-649.

82. Laquiere AE, Laugier RJ, Tuvignon NP: How the Fujinon FICE system may help the endoscopist? *Endoscopy* 2006: **38** (Suppl II) A219.

83. Goetz M, Fottner C, Schirrmacher E et al.: In vivo molecular and subsurface imaging by confocal real time mini-micoscopy. *Endoscopy* 2006: **38** (Suppl II) A215.

84. Wang, TD: Confocal microscopy from the bench to the bedside. *Gastrointest Endosc* 2005: **62**: 696-697.

85. Eberl T, Jechart G, Probst A, et al.: Can an endocytoscope system (ECS) predict histology in neoplastic lesions? *Endoscopy* 2007; **39**: 497-501.

86. Machida H, Sano Y, Hamamoto Y, et al.: Narrow-band imaging in the diagnosis of colorectal mucosal lesions: a pilot study. *Endoscopy* 2004: **36**: 1094-8.

87. Tischendorf JJW, Wasmuth HE, Koch A, et al.: Value of magnifying chromoendoscopy and narrow band imaging (NBI) in classifying colorectal polyps: a prospective controlled study. *Endoscopy* 2007; **39**:1092-1096.

88. Polkowski, M: Endoscopic diagnosis and treatment of upper gastrointestinal tumors. *Endoscopy* 2006: **38**: 1070-1075.

89. Oyama T, Kikuchi Y: Aggressive endoscopic mucosal resection in the upper GI tract: hook knife EMR method. *Min Invas Ther Allied Technol* 2002: **11**: 291-295.

90. Yahagi N, Fujishiro M, Kakushima N, et al: Endoscopic submucosal dissection for early gastric cancer unsing the tip of an electrosurgical snare (thin type). *Dig Endosc* 2004; **16**: 34-38.

91. Yamamoto H, Kawata H, Sunada K, Sasaki A, Nakazawa K, Miyata T, Sekine Y, Yano T, Satoh K, Ido K, Sugano K: Successful en bloc resection of large superficial tumors in the stomach and colon using sodium hyaluronate and small-caliber-tip transparent hood. *Endoscopy* 2003; **35**: 690-694.

92. Yamamoto H: Endoscopic submucosal dissection of early cancers and large flat adenomas. *Clin Gastroenterol Hepatol* 2005; **3** (7Suppl 1): S74-76.

93. Fujishiro M, Yahagi N, Nakamura M, et al: Successful outcomes of a novel endoscopic treatment for GI tumors: endoscopic submucosal dissection with a mixture of high-molecular-weight hyaluronic acid, glycerin, and sugar. *Gastrointest Endosc* 2006; **63**: 243-249.

94. Gotoda T: A large endoscopic resection by endoscopic submucosal dissection (ESD) procedure. *Clin Gastroenterol Hepatol* 2005: **3**: S71-S73.

95. Gotoda T: Endoscopic resection of early gastric cancer: the Japanese perspective. *Curr Opin Gastroenterol* 2006; **22**: 561-569.

96. Kume K, Yamasaki M, Yoshikawa I, Otsuki M: Grasping-forceps-assisted endoscopic submucosal dissection using a novel irrigation cap-knife for large superficial early gastric cancer. *Endoscopy* 2007; **39**: 566-569.

Table 1. Endoscopic tissue stains

STAINS	WHAT IS STAINED?	MECHANISM OF STAINING	POSITIVE STAINING
Absorptive stains		**Absorption into specific cells**	
Lugol's solution	Normal non-keratinized squamous cells	Binds iodine in normal glycogen containing squamous cells	Dark brown
Methylene blue	Small and large intestinal cells or intestinal metaplasia	Active absorption into cells	Blue
Toluidine blue	Nuclei of columnar (gastric and intestinal-type) cells	Diffusion into cells	Blue
Cresyl violet	Margins of colonic mucosal pits	Diffusion into mucosal pits	Purple
Reactive stains		**Identifying cellular products**	
Congo red	Acid secreting mucosa	Color shift at pH less than 3	From red to dark blue or black
Phenol red	H.pylori-infected gastric cells	Color shift at pH 6,8-8,4	From yellow to red
Contrast stain		**Filling surface depressions**	
Indigo carmine	Cells are not stains. Highlights mucosal surfaces	Pools in crevices and valleys between mucosal projections	Blue
Agents for tattooing		**Marking lesions permanently**	
India ink	Injection site	Pooling of carbone in the tissue	Black
Indocyanine green	Injection site	Mark location	green

Table 2. Differentiation from neoplastic - non neoplastic polyps using CE and pit pattern

histology			**accuracy**
predicted	*confirmed*		
Adenomatous polyp (n=64)	adenomatous	hyperplastic	92%
	59	5	
Hyperplastic polyp (n=23)	5	18	78%

Table 3. The morphology and size of 56 lesions treated by EMR

I. protruded type:		20 cases
II.a superficial elevated type:		35 cases
II.a+II.c. superficial elevated and superficial depressed type:		1 case
less than 1 cm in diameter:	27,	from these type I: 4
		type II.a: 22
		type II.a+II.c: 1
1-2 cm in diameter:	26	from these type I: 13
		type II.a: 13
3 cm in diameter	3	all had type I.

Table 4. The differences between the results of biopsies and EMR specimens

	Biopsy (cases)	**EMR specimen** (cases)
Adenoma		
without dysplasia	18	14
with mild dysplasia	2	3
with moderate dysplasia	6	9
with severe dysplasia	7	1
Carcinoma in situ	3	5
Hyperplastic-inflammatory lesion	**18**	**21**
Carcinoid		1
GIST		1
No result because of thermal injury		1

Table 5. The most important differences on histological examination of forceps biopsy and EMR specimens.

FORCEPS BIOPSY	RESECTED SPECIMEN
Hyperplastic polyp	Adenoma with mild dysplasia
Hyperplastic polyp (2 cases)	Adenoma without dysplasia
Hyperplastic polyp	GIST
Adenoma (2 cases)	Hyperplastic polyp
Adenoma with severe dysplasia (4 cases)	Carcinoma in situ
Carcinoma in situ	Carcinoid
Carcinoma in situ	Hyperplastic polyp

Table 6. The results of previous biopsies of proven 17 hyperplastic polyps by EMR histologies

Diagnosis	**number of cases**
Carcinoma in situ	1
Pyogenic granuloma	1
Chronic diffuse gastritis	1
Adenoma	2
Hyperplastic polyp	10
Not known previously	1
Hyperplastic polyp with atypia	1

Table 7. Histological results on forceps biopsy samples and resected specimens in EGC cases

Gender	Age (years)	Location and diameter of lesion	Type of lesion	Forceps biopsy diagnosis	Resected specimen diagnosis
female	70	corpus, 1 cm	IIa	well differentiated intramucosal carcinoma	well differentiated intramucosal carcinoma
female	65	corpus, 1 cm	IIa	well differentiated intramucosal carcinoma	well differentiated intramucosal carcinoma
male	80	antrum, 2 cm	I	well differentiated intramucosal carcinoma	well differentiated neuroendocrine tumor
male	42	antrum, 1 cm	I	hyperplastic polyp	GIST (early)
female	70	corpus, 0.5 cm	IIa	adenoma with high grade dysplasia	well differentiated intramucosal carcinoma
female	73	antrum, 1 cm	I	adenoma with high grade dysplasia	well differentiated intramucosal carcinoma