Enteric nervous system abnormalities in inflammatory bowel diseases
V. Villanacci  G. Bassotti  R. Nascimbeni  E. Antonelli  M. Cadei  S. Fisogni  B. Salerni  K. Geboes

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<td>Ethical considerations</td>
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<td>As this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore no ethical approval was necessary</td>
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**Archival full thickness specimens from 32 IBD patients** (16 from the ileum of CD patients, eight men and eight women, age range 28–47 years; 16 from the colon of UC patients, 10 men and six women, age range 35–52 years) were obtained from patients undergoing surgery for severe disease refractory to medical treatment in the period June 2006–June 2007.

Control specimens were obtained from patients undergoing colonic \( n = 10 \), five men and five women, age range 41–59 years) or ileal \( n = 15 \), 10 men and five women, age range 35–50 years) resection for neoplastic disease. The samples studied were taken at least 3 cm from the resection margin in tumour-free areas. The same methods were used for the evaluation of these control samples.

At least 40 slides (20 from involved and 20 from non-involved areas) for each patient were processed for immunohistochemistry. To evaluate markers of the ENS, we used monoclonal antibodies directed against neuron-specific enolase (NSE, NCL-NSE2, Novocastra laboratories, dilution 1:50; Newcastle upon Tyne, UK) acting as a marker of neuronal cell bodies in the ganglia, and the glial marker protein S100 (S-100, dilution 1:50; Dako, Carpinteria, CA, USA) for enteroglial cells. Since ICC express Kit, an antiKit antibody (CD117, rabbit polyclonal antibody, IgG, dilution 1:50; Dako) was used to detect these cells, as previously reported. The presence of T lymphocytes was assessed by means of monoclonal mouse antihuman CD3 antibody (Dako Cytomation, dilution 1:40). Neuron-specific enolase, S-100 and CD3 immunostaining was carried out using a peroxidase-based visualization kit (Dako LSAB®), following the manufacturer’s recommendations. Diaminobenzidine tetrahydrochloride was used as chromogen. The slides were then counterstained with Mayer’s haematoxylin for 5 s, dehydrated and mounted in Clarion (Biomeda, Foster City, CA, USA). To account for non-specific staining, peptides that blocked polyclonal antibody bindings (passage with normal goat serum) were used, or sections were incubated in the absence of primary antibody. In these cases, no immunostaining was detected.

This is NOT a retrospective study.

The study uses novel material (biopsies) that were specifically prepared for the study.

Thus, the authors have used surgical samples removed from patients and controls (i.e. cancer patients) to cut ex novo at least 40 biopsies from each subject to carry on the staining described in Methods.

These samples were used without patient consent.

Institution NOT indicated
Colonic mast cells in controls and slow transit constipation patients
G. Bassotti, V. Villanacci, R. Nascimbeni, M. Cadei, S. Manenti, G. Sabatino, C. A. Maurer, G. Cantonnier, B. Sartori
First published: 03 May 2011 https://doi.org/10.1111/j.1365-2036.2011.04694.x
Citations: 25
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Diverticular Disease of the Colon: Neuromuscular Function Abnormalities
Gabriella Bassotti, MD, PhD,* Vincenzo Villanacci, MD, Nunzia Bernardini, MD, and Maria P. Dore, MD
DOI 10.1097/MCG.0000000000000578

FIGURE 1. A, Histograms showing the decreased number of interstitial cells of Cajal (I-Kit assessment by CD117) in patients with diverticulosis compared with controls. *Statistically different from controls, B, Histograms showing the decreased number of enteric glial cells (S-100 assessment) in patients with diverticulosis compared with controls. *Statistically different from controls (adapted from Bassotti et al.). C, A representative image of lymphohistiocytic infiltration (CD1 assay) of the myenteric plexus in a patient with diverticular disease; original magnification ×100. D, A representative image of submucosal mast cell infiltration (tryptase assessment) in a patient with diverticular disease; original magnification ×40. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
Enteric nervous system abnormalities in inflammatory bowel diseases. V. Villanacci, G. Bassotti, R. Nascimbeni, E. Antonelli, M. Cadei, S. Fisogni, B. Salerni, K. Geboes

(D) CD3 positive T lymphocytes (arrows) infiltrating the myenteric plexus in a patient with Crohn's disease. Original magnification ×1000.
Figure 2 EGC (arrows) tightly packed around enteric neurons in a submucosal (a) and in a myenteric ganglion (b). S100 immunostaining, original magnifications 40 (a) and 100 (b). Full thickness specimen of human colon of a control subject (c) and of a patient with intractable slow-transit constipation (d). The arrows indicate EGC in the myenteric plexus. Note the rarefaction of these cells in (d). S100 immunostaining, original magnification 10.

Figure 3 (A) Enteric glial cells (arrows) in the myenteric plexus of a patient with ulcerative colitis, uninvolved area. S100, original magnification ×400. (B) Increased number of enteric glial cells (arrows) in an involved area of the same patient. S100, original magnification ×400. (C) Mast cells (arrows) in the muscularis propria of a patient with ulcerative colitis, involved area. CD117, original magnification ×200. (D) Mast cells (arrows) in the muscularis propria of a control. Note that mast cell bodies are more numerous than that seen in C. CD117, original magnification ×200.
Enteric nervous system abnormalities in inflammatory bowel diseases

V. Villanacci, G. Bassotti, R. Nascimbeni, E. Antonelli, M. Cadel, S. Fisogni, B. Salerni, K. Geboes

Published online: 28 August 2008 https://doi.org/10.1136/gut.2008.181449.01.494.e1 Citation: G. Bassotti, Clinica di Gastroenterologia e Epatologia, Ospedale Santa Maria della Misericordia, Piazzale Amerighi, 1, 06136 San Sisto (Pescia), Italy. Tel: +39 075 473 1506; Fax: +39 075 480 7570; e-mail: gabassotti@tin.it

Figure 2 CD117 expression in a control subject (A, B) and in a patient with slow transit constipation (C). Note the decrease in interstitial cells of Cajal (ICC) in the patient's tissue. Original magnifications ×200 (A, C) and ×400 (B). ICC are indicated by arrows. (D) CD34 expression in a patient. Original magnification ×1000.

A  B  C  D

Open in figure viewer PowerPoint

(A) Intestinal cells of Cajal around the myenteric plexus and mast cells in the muscularis propria (arrows) in a patient with Crohn's disease. Note the different shape of mast cells with respect to interstitial cells of Cajal. CD117, original magnification ×200. (B) Intramural interstitial cells of Cajal (arrows) in a patient with Crohn's disease. CD117, original magnification ×200. (C) Intramural interstitial cells of Cajal (arrows) in a control. Note that cell bodies are more numerous than that seen in B. CD117, original magnification ×200. (D) CD34 positive T lymphocytes (arrows) infiltrating the myenteric plexus in a patient with Crohn's disease. Original magnification ×1000.
Figure 2 EGIC (arrows) tightly packed around enteric neurons in a submucosal (a) and in a myenteric ganglion (b). S100 immunostaining, original magnifications 40 (a) and 390 (b). Full thickness specimen of human colon of a control subject (c) and of a patient with intractable slow-transit constipation (d). The arrows indicate EGIC in the myenteric plexus. Note the retraction of these cells in (d). S100 immunostaining, original magnification 10.


10.1097/01.pas.0000213371.79300.a8

Colonic myenteric ganglion of a control, showing a normal number of glial cells (arrows).
The role of colonic mast cells and myenteric plexitis in patients with diverticular disease

Gabrio Bassotti & Vincenzo Villanacci & Riccardo Nascimbeni & Elisabetta Antonelli & Moris Cadei & Stefania Manenti & Luisa Lorenzi & Amin Titi & Bruno Salerni Accepted: 25 July 2012 / Published online: 5 August 2012


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<td>Ethical considerations</td>
<td>Full thickness specimens from the non-inflamed proximal resection margin of 27 patients (12 men and 15 women; age range, 32–87 years) undergoing left hemicolectomy for colonic diverticulitis were retrieved from our archival pathology laboratory. Two groups of patients were evaluated: 12 patients (eight men and four women, aged 59±13 years) undergoing emergency surgery for purulent/faecal peritonitis resulting from free perforation of a diverticulum (Hinchey stages III–IV [18], severe disease according to Ambrosetti classification)</td>
<td>Control samples were obtained from ten patients (five women and five men; age range, 41–78 years) undergoing left hemicolectomy for non-obstructing cancer. These patients were not constipated and had no colonic dilatation. Control specimens were taken at least 3 cm from the resection margin in tumour-free areas.</td>
<td>Methods Full-thickness sigmoid samples were obtained from formalinfixed tissue and transversal sections obtained after paraffin embedding were processed for both conventional histology (H&amp;E) and immunohistochemistry (IHC). A specific antibody targeting MC tryptase [22] (monoclonal mouse clone 10D11, dilution 1:200, Novocastra, UK) was used. Paraffin sections were dewaxed and rehydrated through decreasing alcohol series up to distilled water. Sections were then subjected to heat-induced epitope retrieval by immersion in a heat-resistant container filled with citrate buffer solution (pH 6.0) placed in a pressure cooker and microwaved at 95 °C for 20 min. Endogenous peroxidase activity was suppressed by incubation with 3 % solution of H2O2 for 5 min. In patients with MC degranulation (see below) double immunohistochemistry (developing the neural marker neuronspecific enolase (NSE) NCL-NSE2, Novocastra laboratories, dilution 1:50, with a red chromogen) was used to evaluate the relationship between MC and nerve fibres in the mucosa. The presence of T lymphocytes was assessed by IHC.</td>
<td>This is NOT a retrospective study. The study uses novel material (biopsies) that were specifically prepared for the study. Thus, the authors have used surgical samples removed from patients and controls (i.e. cancer patients) to cut ex novo at least 40 biopsies from each subject to carry on the staining described in Methods. These samples were used without patient consent. Institution NOT indicated</td>
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[19]) and 15 patients (4 men and 11 women, aged 64±12 years) undergoing elective surgery after the third or fourth attack of diverticulitis [20, 21].

| Images in this paper | using the monoclonal mouse anti-human CD 3 antibody (Dako Cytomation, dilution 1:40). |  |
**Colonic mast cells in controls and slow transit constipation patients**

G. Bassotti  V. Villanacci  R. Nascimbeni  M. Cadei  S. Manenti  G. Sabatino  C. A. Maurer  G. Cathomas  B. Salerni


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<td>Ethical considerationsAs this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore a simplified IRB approval was obtained.</td>
<td>PATIENTSSurgical full-thickness specimens from 29 patients (24 women, 5 men, age range 27–75 years) undergoing subtotal colectomy with ileorectostomy for severe intractable constipation were evaluated. Twenty of these patients have previously been investigated in a study on enteric nervous system (ENS) abnormalities.18</td>
<td>As right and left sections of the colon were analysed in patients, for caecum, ascending and transverse we obtained control samples from 10 patients (eight women, two men, age range 38–70 years) undergoing right hemicolecctomy and for descending and sigmoid from 10 patients (six women, four men, age range 41–78 years) undergoing left hemicolecctomy, both for non-obstructing cancer. These patients were not constipated and had no colonic dilatation. At the time of surgery, the patients were prepared with polyethylene glycol; three subjects used antihypertensive drugs (two lisinopriland one propranolol). The control specimens were taken at least 3 cm from the resection margin in tumour free areas.</td>
<td>Tissues were fixed in 10% neutral-buffered formalin for 24 h, then full-thickness samples from multiple colonic segments (cecum, ascending, transverse, descending and sigmoid areas) were taken and transversal sections obtained. Sections were processed for both conventional histology (H&amp;E and Trichrome stain) and immunohistochemistry (IHC). MC detection A specific antibody targeting MC tryptase20 (monoclonal mouse clone 10D11, dilution 1:200, Novocastra, UK) was used. Paraffin sections were dewaxed and rehydrated through decreasing alcohol series up to distilled water. The sections were then subjected to heat-induced epitope retrieval by immersion in a heat-resistant container filled</td>
<td>This is NOT a retrospective study. The study uses novel material (biopsies) that were specifically prepared for the study. These samples were used without patient consent. Institution NOT indicated NOTE this study mention that some of the patients were studied in ref. 18. Reference 18 is a paper from GUT. See below</td>
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Images in this paper

Colonic mast cells in controls and slow transit constipation patients
G. Bassotti, V. Villanacci, R. Nascimbeni, M. Cadei, S. Manenti, G. Sabatino, C. A. Maurer, G. Cattoni, B. Salerni
Prof. G. Bassotti, Gastroenterology & Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, Piazza Menghini, 1, 06156 San Sisto (Perugia), Italy.

Diverticular Disease of the Colon: Neuromuscular Function Abnormalities
Gabriol Bassotti, MD, PhD,* Vincenzo Villanacci, MD, Nunzia Bernardini, MD, and
Maria P. Dore, MD. Doi 10.1097/MCG.0000000000000578

**Slow Transit Constipation**

**Diverticular disease**

**FIGURE 1.** A, Histograms showing the decreased number of interstitial cells of Cajal (c-kit assessment by CD117) in patients with diverticulosis compared with controls. *Statistically different from controls. B, Histograms showing the decreased number of enteric glial cells (5-100 assessment) in patients with diverticulosis compared with controls. *Statistically different from controls (adapted from Bassotti et al.1). C, A representative image of lymphocytic infiltration (CD3 assessment) of the myenteric plexus in a patient with diverticular disease; original magnification x 100. D, A representative image of submucosal mast cell infiltration (tryptase assessment) in a patient with diverticular disease; original magnification x 40. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
Representative images of MC in Slow Transit Constipation.

Diverticular disease
The Role of Glial Cells and Apoptosis of Enteric Neurones in the Neuropathology of Intractable Slow Transit Constipation

G Bassotti, V Villanacci, C A Maurer, S Fisogni, F Di Fabio, M Cadei, A Morelli, T Panagiotis, G Cathomas, B Salerni

Type study according to the Materials and methods

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| Twenty six STC patients (25 women, one man; age range 24–78 years) undergoing colectomy with ileorectostomy for severe intractable constipation were enrolled in the study. Inclusion criteria were: (1) longstanding history of constipation (more than three years; mean 14 (range 3–59)); symptoms arose in childhood in one patient and in later life in the others; (2) one or fewer evacuations per week; (3) absence of frequent (more than two episodes per month) or chronic abdominal pain; (4) sensation of incomplete evacuation in >1/4 defecations; (5) negative history for (sub)occlusive episodes; and (6) unresponsiveness to appropriate and intensive medical treatment, including high fibre diet, stimulant and osmotic laxatives, and enemas. Intestinal transit time, measured by means of radiopaque markers, was delayed in all patients (up to more than 240 hours). Causes of secondary constipation were excluded by drug history, physical examination, and laboratory screening (blood chemistry, thyroid hormones and, where appropriate, oral glucose tolerance test, sex hormone profiles, and antinuclear antibodies). To exclude organic diseases or
| Ten patients (nine women, one man; age range 43–75 years) undergoing left hemicolecstomy for non-obstructing colorectal cancer were used as controls as there is evidence that the distribution of ICC is relatively uniform throughout the human colon. | After removal, surgical specimens were immediately fixed in 10% neutral buffered formalin for 24 hours, and then 12–20 full thickness samples from the whole excised colon were taken and transversal sections obtained. For conventional histology, 5 µm paraffin sections were stained with haematoxylin-eosin, periodic acid-Schiff (PAS), and trichrome stain. Immunohistochemistry
| After removal, surgical specimens were immediately fixed in 10% neutral buffered formalin for 24 hours, and then 12–20 full thickness samples from the whole excised colon were taken and transversal sections obtained. For conventional histology, 5 µm paraffin sections were stained with haematoxylin-eosin, periodic acid-Schiff (PAS), and trichrome stain. Immunohistochemistry
| At least 40 slides for each patient were processed for immunohistochemistry. To evaluate markers of the enteric nervous system we used monoclonal antibodies towards neurone specific enolase (NSE, NCL-NSE2, dilution 1:50; Novocastra Laboratories, Newcastle upon Tyne, UK) acting as a marker of gangliar cells, and the glial marker protein S100 (S-100, dilution 1:50; Dako, Carpinteria, California, USA) was used. As ICC express Kit, an anti-Kit antibody (rabbit |

This is NOT a retrospective study. The study uses novel material (biopsies) that were specifically prepared for the study. Thus, the authors have used surgical samples removed from patients and controls (i.e. cancer patients) to cut ex novo at least 40 biopsies from each subject to carry on the staining described in Methods. These samples were used without patient consent. Institution NOT indicated
mechanical causes of constipation and megacolon or megarectum, each patient underwent double contrast barium enema and/or colonoscopy. Absence of Hirschsprung's disease was demonstrated by normal relaxation of the internal anal sphincter at anorectal manometry. No patient had evidence of obstructed defecation, as documented by anorectal manometry and/or defecography.

Polyclonal antibody, IgG, dilution 1:50; Dako) was used to detect these cells, as previously reported. Moreover, CD34 staining (CD34 clone QBEnd/10, dilution 1:30; Neo markers, Union City, California, USA) was used to evaluate the population of fibroblast-like cells which are intimately associated with the ICC. Two methods were used as markers for apoptosis in the enteric nervous system: (a) expression of Bcl-2 protein (BCL2 oncoprotein clone 124, dilution 1:10; DBS, Pleasantown, Australia), a
Enteric glial cells and their role in gastrointestinal motor abnormalities: Introducing the role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation

Gabriele Bassotti, Vincenzo Villanacci, Simona Fisogni, Elisa Rossi, Paola Baronio, Carlo Clerici, Christoph A Maurer, Gieri Cathomas, and Elisabetta Antonelli


G Bassotti, V Villanacci, C A Maurer, S Fisogni, F Di Fabio, M Cadei, A Morelli, T Panagiotis, G Cathomas, and B Salerni
The enteric nervous system in patients with calculus and acalculus gallbladder
Vincenzo Villanacci a Rachele Del Sordo Marianna Salemme Moris Cadei Angelo Sidoni Gabriele Bassotti

https://doi.org/10.1016/j.dld.2016.03.014
Digestive and Liver Disease
Volume 48, Issue 7, July 2016, Pages 792-795

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### Ethical considerations
Dealing with a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore, no ethical approval was necessary.

### Materials and methods
Archival samples of surgically excised gallbladders were obtained from 39 patients, 27 with cholesterol gallstones (7 men, 20 women, median age 53, range 45–69 yrs) and 12 patients without gallstones (5 men, 7 women, median age 5, range 39–71 yrs). Full-thickness samples were obtained from formalin fixed tissue and transversal sections obtained from the neck of the gallbladder after paraffin embedding and processed for both conventional histology (H&E) and immunohistochemistry (IHC). The neck area was chosen on the basis of previous studies showing that this part of the gallbladder features the higher number of nerve cells [9], [10].

Concerning the various elements of the ENS, enteric neurons were assessed by both neuron-specific enolase (NSE, monoclonal antibody clone 22C9, Leica Microsystems Srl, Milano, Italy; dilution 1:200) and calretinin (polyclonal antibody, Histo-Line Laboratories, Pantigliate, Milano, Italy; dilution 1:250), the enteric glial cells (EGC) by S100 (polyclonal antibody, Leica Microsystems Srl, Milano, Italy; dilution 1:300), and the ICC by CD117 (polyclonal antibody, Dako, Carpinteria, CA; dilution 1:200). To distinguish ICC from mast cells, sections were also assessed by tryptase (monoclonal antibody clone AA1, Bio-Optica, Milano, Italy; dilution 1:4000). Moreover, to further characterize ICC and ICC-like cells, the so called telocytes [11], sections were also stained with CD34 (monoclonal antibody clone Qbend/10, Leica Microsystems Srl, Milano, Italy; dilution 1:200) which helps to identify this cell population [12]. For each patient, the number of immunopositive cells was calculated and expressed as the mean of cells on 10 well stained and well oriented microscopic fields.

This is NOT a retrospective study.

The study uses novel material (biopsies) that were specifically prepared for the study.

Thus, the authors have used surgical samples removed from patients and controls (i.e. cancer patients) to cut ex novo at least 40 biopsies from each subject to carry on the staining described in Methods.

These samples were used without patient consent.

Institution NOT indicated.
fields for each region of interest at 40× magnification (Olympus BX 40, Tokyo, Japan). The slides were coded to ensure anonymity, and all calculations were made in blind by one of the authors unaware of the diagnosis.

| Images in this paper | NONE DUPLICATED |
Optimal processing of ESD specimens to avoid pathological artifacts
L. Reggiani BonettiEmail author R. Manta M. Manno R. Conigliaro G. Missale G. Bassotti V. Villanacci

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<td>Ethical issues</td>
<td>The study included 53 en bloc ESD specimens retrospectively collected from the archives of the Institute of Pathology of Modena and the Institute of Pathology of Brescia during a 5-year period (2000–2005). All patients were referred for UGI or LGI tract ESD after the endoscopic detection of mucosal lesions, endoscopically classified according to the Paris criteria [8].</td>
<td>Forty endoscopically resected specimens were gently posed on a cellulose board, then put into a special fenestrated boxes (biocassettes for mucosectomy, Bioptica®, Milan, Italy) and covered with a thin sponge, as previously described [2, 7] (Fig. 1). The boxes were immersed in 10% neutral-buffered formalin for 24 h fixation.</td>
<td>THIS PAPER IS A FAKE. THE BIOPTIC INSTRUMENTS WAS DESCRIBED FIRST BY THESE AUTHORS IN 2012 REFERENCE 2 AND 7.. SO IT IS IMPOSSIBLE TO HAVE USED IT FOR SAMPLES COLLECTED IN 2000-2005!</td>
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Gastrointestinal Foxp3 expression in normal, inflammatory and neoplastic conditions

VINCENZO VILLANACCI*, TARCISIO NOT{, RICCARDO NASCIMBENIz, FORTUNATO FERRARA{, ALBERTO TOMMASINI{, STEFANIA MANENTI*, ELISABETTA ANTONELLI§ AND GABRIO BASSOTTI§

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<td>MATERIALS AND METHODS Overall, 216 samples of gastrointestinal tissues were obtained from 200 patients. They were enrolled consecutively at the same pathology laboratory during 2009–2010 according to their disease and gastrointestinal biopsy/specimen site. No exclusion criteria were applied. The samples were included in the following disease/site groups: 1. Ten cases of oesophagitis (8 cases of reflux oesophagitis, and 2 cases of eosinophilic oesophagitis). 2. Ten cases of chronic active Helicobacter pylori positive gastritis, classified according to Sidney21 and OLGA22 systems, with a minimum of five biopsies obtained from antrum, angulus and corpus. 3. Ten cases of Helicobacter pylori negative, microscopically normal gastric samples, biopsied as above and obtained during upper GI endoscopy as part of a diagnostic study protocol.</td>
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<td>The study protocol was approved by the institutional review board, even if it provided for the totally anonymous use of tissue blocks so that the informed consent of the patient was not deemed mandatory.</td>
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The primary endpoint of the study was the number of Foxp3⁺ cells per field as measured by immunohistochemistry. Further immunohistochemical assessments comprised the count of CD3, CD4, and CD8 T cells. The proportion of Foxp3⁺ cells was calculated on the percentage of CD3 cells expressing the Foxp3 epitope. Beside the primary digestive disease categorisation cited above, age and gender of patients, and the gastrointestinal site of sample withdrawal were recorded and analysed. All cases (endoscopic biopsies or surgical specimens) were processed to detect CD3 and Foxp3⁺ cells by immunohistochemical methods; CD4 and CD8 T cells were evaluated only in cases of coeliac disease, at the time of the diagnosis and after the gluten-free diet. Formalin fixed paraffin sections were dewaxed and rehydrated through decreasing
work-up for upper abdominal symptoms. They were used as gastric control group.

4. Twenty-one cases of gastric carcinoma, classified according to Lauren into nine diffuse-type carcinomas, and 13 intestinal-type carcinomas [UICC classification: stage I (n=2), stage II (n=6), stage III (n=13)].

5. Forty-nine cases of active coeliac disease, classified according to MarshOberhuber and New Grading System,23,24 with different degrees of atrophy in all cases. Further samples were obtained from 16 cases of the previous 49 after being on a gluten-free diet for 12–18 months.

6. Ten cases of microscopically normal duodenal mucosa obtained during upper GI endoscopy as part of a diagnostic work-up for upper abdominal symptoms, and used as control group of coeliac disease.

7. Twenty-one cases of adenomatous polyps of the colon, 12 with low-grade dysplasia, and nine with high-grade dysplasia.

8. Thirty-eight cases of colon carcinoma [UICC classification: stage I (n=5), stage II (n=15), stage III (n=18)].

9. Ten cases of ulcerative colitis, seven with histologically active disease and the remaining three with quiescent disease.

10. Ten cases of alcohol series up to distilled water. Sections were then subjected to heat-induced epitope retrieval by a EDTA solution (pH 8.0) at 98°C for 40 min. Endogenous peroxidase activity was suppressed by incubation with 3% solution of H2O2 for 5 min. The following antibodies were employed, using commercially available kits and following the manufacturer’s recommendations: Foxp3 (PCH 101, dilution 1:660; Bioscience, USA), CD3 (1:250; Neomarkers, USA), CD4 (1:50; Neomarkers), CD8 (1:100; Dako, Denmark), CD20 (clone L26, dilution 1:100; Dako). The number of positive cells was counted for each patient in 10 high power fields (HPF, 40) by two pathologists (VV, SM).
Crohn's disease, five with histologically active disease and five with quiescent disease. 11. Ten cases of microscopically normal colonic mucosa from asymptomatic patients with incidental diagnosis of diverticulosis during screening colonoscopy, used as colonic control group.
Enteric neuroglial apoptosis in inflammatory bowel diseases
Gabrio Bassotti, Vincenzo Villanacci, Riccardo Nascimbeni, Moris Cadei, Simona Fisogni, Elisabetta Antonelli, Nadia Corazzi, Bruno Salerni

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<td><strong>3.4 Ethical considerations</strong></td>
<td>2 Patients and methods</td>
<td>Control specimens were obtained from patients undergoing colonic (n = 10, 5 men and 5 women, age range 41–59 years) or ileocolonic (n = 15, 10 men and 5 women, age range 35–50 years) resection for neoplastic disease. No control had received treatments likely to alter the ENS. The samples studied were taken at least 3 cm from the resection margin in tumour free areas. The same staining methods used for the patients were also used for the evaluation of these control samples. The time from tissue resection to fixation was similar for both patients and controls.</td>
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<td>Archival full thickness specimens from 19 IBD patients (9 from the ileum of CD patients, 5 men and 4 women, age range 37–49 years; 10 from the colon of UC patients, 5 men and 5 women, age range 43–57 years) were obtained from patients undergoing surgery for severe disease refractory to medical treatment in the period July 2007–January 2008. The diagnosis of CD or UC was based on clinical, radiologic, and endoscopic examination and histologic findings. All IBD patients had been treated with 5-amino-salicylic acid and immunosuppressive drugs.</td>
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<td><strong>3.1 Immunohistochemistry</strong></td>
<td><strong>Immunohistochemistry</strong></td>
<td>At least 20 slides for each patient were processed for immunohistochemistry. We used monoclonal antibodies directed against neuron-specific enolase (NSE, NCL-NSE2, Novocastra laboratories, dilution 1:50) as a marker of neuronal cell bodies in the ganglia, and the glial marker protein S100 (S-100, Dako, dilution 1:50) for enteroglial cells, as previously described. Apoptosis was evaluated with two methods: by evaluating the expression the caspase-3 (a so-called executioner caspase), and by a monoclonal antibody to single-stranded DNA, using the formamide monoclonal antibody (formamide-MAb) method, as previously described. NSE and S-100 immunostaining was carried out, as previously described.</td>
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Again this paper uses surgical samples from patients with colitis and control subjects (colon cancer patients) without patient consent and authorization by ethical committee.
The samples studied were taken from macroscopically involved areas. For each patient we chose the samples better oriented and more representative of disease described,17–20 using a peroxidase-based visualization kit (Dako LSAB®), following the manufacturer's recommendations. Diaminobenzidine tetrahydrochloride was used as chromogen. The slides were then counterstained with Mayer's hematoxylin for 5 s, dehydrated and mounted in Clarion (Biomeda). To account for non-specific staining, peptides that blocked polyclonal antibody bindings (passage with normal goat serum) were used, or sections were incubated in th.
Interstitial cells of Cajal, enteric nerves, and glial cells in colonic diverticular disease


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<td>Patients</td>
<td>Controls</td>
<td>Munohistochemistry</td>
<td>Agaion this paper reports on the use of surgical samples from patients with diverticuklar disease and control subjects (colon cancer) with no patients consnet</td>
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Patients
Colon specimens were obtained from 39 patients (17 men, 22 women; age range, 49–78 years) undergoing elective left hemicolecctomy for diverticular disease (35 cases) or emergency surgery for acute diverticulitis with periolic abscess (four cases) in the period January 1999 to January 2004. None of the patients had concomitant tumours, bowel obstruction, or other diseases of the colon.

Methods
We used 10 specimens from age and sex matched subjects undergoing left hemicolecctomy for non-obstructing colorectal cancer as controls. The control specimens were taken at least 5 cm from the resection margin in tumour free areas.

munohistochemistry
At least 10 samples (five from diverticular and five from macroscopically normal portions) for each patient were processed for immunohistochemistry. To evaluate the enteric nervous system, we investigated PGP 9.5 (protein gene product 9.5), a cytoplasmic protein that acts as a marker of general neural tissue, and the glial marker protein S-100. Ganglion cells were assessed with a monoclonal anti-PGP 9.5 antibody (IgG2a; 1/100 dilution; Biomeda, Foster City, CA).
Schwann cells, intragangliar glial cells close to ICC, and myenteric ganglia were assessed with a specific monoclonal antibody (anti-S-100; 1/50 dilution; Dako, Carpinteria, California, USA). S100 immunostaining highlights ganglion cells as prominent negatively stained cells surrounded by positive Schwann/glial cells.
An assessment of enteric nervous system and estroprogestinic receptors in obstructed defecation associated with rectal intussusception

Gabrio Bassotti  Vincenzo Villanacci  Alberto Bellomi  Rossella Fante  Moris Cadei  Luca Vicenzi  Francesco Tonelli  Gabriella Nesi  Corrado R Asteria
First published:21 December 2011

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<td>We retrospectively analyzed full-thickness rectal specimens from a series of patients with OD undergoing STARR for symptoms unresponsive to conventional measures (including lifestyle changes, dietary manipulation, laxatives, and biofeedback)34 undergoing surgery in the period September 2007–September 2009. All patients fulfilled Rome II criteria for constipation (i.e., two or more of six symptoms present for at least 12 weeks of the preceding 12 months: straining, lumpy, or hard stools, sensation of incomplete evacuation, sensation of anorectal obstruction/blockage, or manual maneuvers to facilitate defecation on more than one-fourth of bowel movements, or</td>
<td>Rectal tissue from ten patients [eight women, two men, aged 61 (54–70) years], undergoing rectal resection for cancer, was obtained. Sections were taken at least 3 cm from the neoplasms, and the margins were ascertained to be tumor-f</td>
<td>Tissue samples were processed as previously described.28, 31, 38, 39 After removal, the surgical specimens were immediately fixed in 10% neutral-buffered formalin for 24 h and transversal sections obtained. For conventional histology 5-μm paraffin sections were stained with Hematoxylin-Eosin, Pas, and Trichrome stain. At least 10 slides for each patient were processed for immunohistochemistry (IHC). To evaluate markers of the ENS, monoclonal antibodies toward neuron-specific enolase (NSE, NCL-NSE2, dilution 1:50; Novocastra Laboratories, Newcastle upon Tyne, UK) acting as a marker of gangliar cells, and the glial marker protein S100 (S-100, dilution 1:50; Dako, Carpinteria, CA, USA) were used. For the interstitial cells of Cajal (ICC) an anti-Kit antibody (rabbit polyclonal</td>
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<td>Ethical considerations</td>
<td>This paper uses again surgical samples for ex novo studies without informed consent from patients and control (colon cancer patients).</td>
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As this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore no ethical approval was necessary.
| less than three evacuations per week), colonoscopy was normal, and adequate external sphincter function on rectal examination and evidence of rectal intussusception and/or anterior rectocele on dynamic defecography were present in all patients. A validated constipation score for defecation disorders was also available. The surgical procedure was carried out according to the method described by Boccasanta and colleagues.37 |
| antibody, IgG, dilution 1 : 50, Dako) was used. The NSE and S-100 immunostaining was carried out using a peroxidase-based visualization kit (Dako LSAB®), following the manufacturer’s recommendations. Diaminobenzidine tetrahydrochloride was used as chromogen. The slides were then counterstained with Mayer’s hematoxylin for 5 s, dehydrated and mounted in Clarion (Biomedia, Foster City, CA, USA). |

Images in this paper
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### Fibrotic and Vascular Remodelling of Colonic Wall in Patients with Active Ulcerative Colitis

Chiara Ippolito, Rocchina Colucci, Cristina Segnani, Mariella Errede, Francesco Girolamo, Daniela Virgintino, Amelio Dolfi, Erika Tirotta, Piero Buccianti, Giulio Di Candio ... Show more


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<td>The selection of UC and control patients was based on the availability of both frozen and paraffin-embedded colonic tissues archived in the pathology tissue bank. Since the study was performed on archival material, no individual patient identification was involved, and no study-driven clinical intervention was performed. Accordingly, a simplified procedure for Institutional Review Board approval was followed. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.</td>
<td>Full-thickness samples of left [descending and sigmoid] colon were retrieved from UC patients with active, pharmacologically unresponsive disease, without clinical symptoms of fibrostenosis, who had undergone elective bowel resection due to a severe exacerbation of colitis. Based on the disease duration after the UC diagnosis [colectomy within 3 years or after 10 years], patients were allocated to two subgroups, respectively designated as SL [ n = 9, 6 males and 3 females, age range 22–74 years] and LL [ n = 10, 5 males and 5 females, age range 41–77 years] U</td>
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<td>This paper report the acquisition of IRB but the institution that released the IRB is not reported. Further, authors are from several institutions. Additionally, the Oviedo Convention clearly states that the patients’ consent can NOT be omitted and IRB is NOT a substitute for the informed consent.</td>
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### Ethical considerations

Dealing with a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore no ethical approval was necessary.

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| Archival slides from 83 cases (50 men and 35 women, aged 51.5 ± 9.4 years) of colonic biopsies obtained in patients undergoing a first endoscopic assessment for a clinical suspicion of IBD (and free from treatments) were retrospectively reviewed in two different centers. The slides were collected from the Pathology department (Center 1) of Spedali Civili di Brescia and from the Pathology department (Center 2) of Azienda Ospedaliera Città della Salute e Della Scienza, Torino. Endoscopic data, microscopic pattern with IBD-related lesions (ulcerations, granulocyte exocytosis, and crypt distortion) and the final diagnosis were used to identify the cases.

In order to better assess the most suitable antibody for plasma cells staining the sections were stained with two monoclonal antibodies against plasma cells (CD138, clone M15 – Dako, Glostrup, Denmark 1:70, and MUM 1 clone MUM1p Dako, Glostrup, Denmark, 1:80). CD 138 stains cytoplasmic membrane of plasma cells and epithelial cells, while MUM-1 specifically stains plasma cells membrane. Eosinophils were stained with CD193 (clone Y31, Abcam, Cambridge, UK, 1:75). The labeling system used were Bond™ Polymer Refine Detection (MUM-1 and CD138), and Bond Polymer Refine Red Detection Novocastra (CD193 – Leica BOND-MAX stainer, Leica Biosystems, Milano, Italy).

This paper uses endoscopic biopsies for ex novo studies without informed consent from patients.
Histologic diagnosis were obtained for all cases.

Images in this paper
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Myenteric plexitis: A frequent feature in patients undergoing surgery for colonic diverticular disease

Gabrio Bassotti, Vincenzo Villanacci, Angelo Sidoni, Riccardo Nascimbeni, Maria P Dore, Gian A Binda, Roberto Bandelloni, Marianna Salemme, Rachele Del Sordo, Moris Cadei, Alessandra Manca, Nunzia Bernardini, Christoph A Maurer, Gieri Cathomas

First Published December 18, 2014 Research Article Find in PubMed
https://doi.org/10.1177/2050640614563822

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<tr>
<td>Ethical considerations</td>
<td>Patients and controls We retrieved resection specimens of patients with colonic diverticulitis from four archival pathology laboratories in Italy (in Brescia, Genova, Perugia and Sassari) and one in Switzerland (Liestal). Patients were subdivided in two groups, i.e. patients undergoing emergency surgery for purulent/fecal peritonitis, resulting from free perforation of a diverticulum (Hinchey Stage III–IV, 23 severe disease according to Ambrosetti classification) and patients undergoing elective surgery after either the third or fourth attack of diverticulitis or for sigmoid stenosis, due to recurrent episodes of diverticulitis.</td>
<td>We obtained control samples from the proximal resection margin of 15 patients (seven women and eight men; age range 44–83 years) undergoing left hemicolecotomy for non-obstructing cancer. These patients were not constipated nor colon-dilated. Control specimens were taken at least 3 cm from the resection margin, from tumor-free areas.</td>
<td>Methods</td>
<td>This paper uses again surgical samples for ex novo studies without informed consent from patients (diverticular disease) and control (colon cancer patients). Four centers were involved: brescia, genova, Perugia and Sassari. NOTE that antiCD3 staining is not a part of the workup for diverticular disease or colon cancer</td>
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full-thickness sigmoid samples from formalin-fixed tissue and obtained transversal sections after paraffin embedding and processing for both conventional histological hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC). We assessed the presence of T lymphocytes by IHC, using the monoclonal mouse anti-human CD3 antibody (Dako Cytomation, Carpinteria, CA, USA) at a dilution of 1:40.
**Epidermal growth factor receptor overexpression/amplification in adenocarcinomas arising in the gastrointestinal tract**
Elisa Rossi 1,2, Vincenzo Villanacci 1, Cesare Danesino3, Francesco Donato4, Riccardo Nascimbeni 5 and Gabrio Bassotti 6

*REV ESP ENFERM DIG (Madrid) Vol. 103. N.° 12, pp. 632-639, 2011*
http://dx.doi.org/10.4321/S1130-01082011001200005

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<td>There is no mention to any ethical consideration or consent</td>
<td>Sixty-four tumor specimens were evaluated: 17 adenocarcinomas arising in Barrett’s esophagus, 21 stomach cancers (6 diffuse type, 14 intestinal type, 1 intestinal type with mucoid differentiation), 17 colon cancers (14 moderatelypoorly differentiated adenocarcinomas, 3 mucoid) and 9 liver metastasis of colon carcinoma (2 cases were metastasis of above colon cancer and 7 were from different cases).</td>
<td></td>
<td>Immunohistochemistry EGFR (HER1) receptor status was analyzed by the EGFR pharmDx kit (DAKOCytomation, Carpinteria, CA, USA). Fluorescence in situ hybridization (FISH) EGFR is located on chromosome 7p12 and in FISH it is investigated by a LSI © Locus Specific Identifier DNA Probe labeled by Spectrum Orange fluorochrome (VysisInc., Downers Grove, IL, USA). The LSI © probe consists of DNAprobe sequences homo</td>
<td>This paper uses surgical samples from 64 cancers patients for ex novo studies without informed consent. IRB is not mentioned</td>
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Expression of the Rai (Shc C) adaptor protein in the human enteric nervous system

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<td>Ethical considerations As no individual patient identification was involved and no study-driven clinical intervention was performed, a simplified Institutional Review Board approval was obtained and no patient consent was considered necessary</td>
<td>Archival histological sections from human gastrointestinal tract (oesophagus, three subjects; stomach, five subjects; small bowel, five subjects; colon, eight subjects) were derived from adult patients undergoing surgery for cancer in the month prior to the start of the study. The sections were obtained at least 3–5 cm from the resection margin in tumour-free areas. The colonic specimens were taken from both the right (n = 5) and the left (n = 3) colon.</td>
<td></td>
<td>Double immunochemistry (developing the anti-Rai with a red chromogen) was used to simultaneously reveal the interstitial cells of Cajal (ICC), identified by means of an anti-Kit antibody (rabbit polyclonal antibody, IgG; Dako, Carpinteria, CA, dilution 1 : 50), as previously described.24,25 Immunohistochemistry for EGC was performed using antibodies against the specific markers S100 (rabbit polyclonal antibody S-100; Dako, dilution 1 : 50) and glial fibrillary acidic protein, GFAP.</td>
<td>This paper uses again surgical samples for ex novo studies without informed consent from patients. Several centers were involved. Brescia, Milan, Perugia, Liestal (Switzerland). The institution that released the IRB is not mentioned. Animal ethical committee is not described. Myenteric Myenteric</td>
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One-shot balloon dilation of esophageal stricture due to unusual lichen planus localization. Mariano Sica, Claudio Zulli, Raffaele Manta, Vincenzo Villanacci, Rita Conigliaro, Gabrio Bassotti  Journal of gastrointestinal and liver diseases : JGLD (2016) Gastroenterology & Hepatology Section, Department ...

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Is pseudomelanosis coli a marker of colonic neuropathy in severely constipated patients?  

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<td>The studies were carried out in accordance with local ethical guidelines, following the recommendations of the Declaration of Helsinki (Edinburgh revision, 2000).</td>
<td>Patients and methods Specimens were examined from 16 female patients (age range 24–77 years) fulfilling the Rome II criteria for constipation undergoing colectomy with ileorectostomy for intractable STC, and in whom the presence of PMC was demonstrated both at colonoscopy and at conventional histological examination. PMC in these</td>
<td>.</td>
<td>Immunohistochemistry At least 20 slides for each patient were processed for immunohistochemistry. The monoclonal mouse antihuman macrophage antibody (CD68, clone KP1; Dako, Carpinteria, CA, USA; dilution 1 : 50) was used to confirm that the PMC cells observed at conventional</td>
<td>This paper uses again surgical samples for ex novo studies without informed consent from patients who their colon resected for constipation. NOTE that this is a very uncommon indication for colon resection. Why the informed consent for histology studies was not</td>
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patients was thought to be due to the ingestion of anthraquinone laxatives, since all of them had used such compounds (mostly the senna-containing over-the-counter drug Pursennid) for an average of 8 years (range 4–12). There was no history of chronic use of other drugs known to cause PMC or of non-steroidal anti-inflammatory drugs. These patients were part of a study on enteric nervous system abnormalities and were shown to have a significant decline in enteric neural structures, particularly gangliar neurons (which also displayed an increased number of apoptotic cells) and ICC, compared with controls. Histological and immunohistochemical evaluations were carried out according to previously described techniques. After removal, the surgical specimens were immediately fixed in 10% neutral-buffered formalin for 24 h, after which histology were indeed macrophages (Figures 2A). There is concern over the indication for surgery.

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<td>We retrospectively analyzed full-thickness rectal specimens from a series of patients with OD undergoing STARR for symptoms unresponsive to conventional measures (including lifestyle changes, dietary manipulation, laxatives, and biofeedback) 34 undergoing surgery in the period September 2007–September 2009</td>
<td><strong>Controls</strong> Rectal tissue from ten patients [eight women, two men, aged 61 (54–70) years], undergoing rectal resection for cancer, was obtained. Sections were taken at least 3 cm from the neoplasms, and the margins were ascertained to be tumor-free.</td>
<td>Tissue samples were processed as previously described. After removal, the surgical specimens were immediately fixed in 10% neutral-buffered formalin for 24 h and transversal sections obtained. For conventional histology 5-lm paraffin sections were stained with Hematoxylin-Eosin, Pas, and Trichrome stain. At least 10 slides for each patient were processed for immunohistochemistry (IHC). To evaluate markers of the ENS, monoclonal antibodies toward neuron-specific enolase (NSE, NCL-NSE2, dilution 1 : 50; Novocastra Laboratories, Newcastle upon Tyne, UK) acting as a marker of gangliar cells, and the glial marker protein S100 (S-100, dilution 1 : 50; Dako, Carpinteria, CA, USA) were used. For the interstitial cells of Cajal (ICC) an anti-Kit antibody (rabbit polyclonal antibody, IgG, dilution 1 : 50, Dako) was used.</td>
<td>Surgical samples for ex novo studies without informed consent from patients (constipation) and control (rectal cancer patients).</td>
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**Ethical considerations**
As this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore no ethical approval was necessary.
| The NSE and S-100 immunostaining was carried out using a peroxidase-based visualization kit (Dako LSAB_), following the manufacturer’s recommendations. Diaminobenzidine tetrahydrochloride was used as chromogen. The slides were then counterstained with Mayer’s hematoxylin for 5 s, dehydrated and mounted in Clarion (Biomeda, Foster City, CA, USA). To account for non-specific staining, peptides that blocked polyclonal antibody bindings (passage with normal goat serum) were used, or sections were incubated in the absence of primary antibody. In these cases, no immunostaining was detected. Expression of Kit: consecutive formalin-fixed, paraffin sections were dewaxed and rehydrated through decreasing alcohol series up to distilled water. Sections were then subjected to heat-induced epitope retrieval by immersion in a heat-resistant container filled with citrate buffer solution (pH 6.0) placed in a pressure cooker and microwaved for 20 min. Endogenous peroxidase activity was suppressed by incubation with 3% solution of H2O2 for 5 min. Kit immunostaining was carried out using a peroxidase-based visualization kit (Dako EnVision_; Dako, Carpinteria, CA, USA), following the manufacturer’s recommendations. Kit-positive mast cells served as internal control. |
Concerning the estrogen–progesterone receptors, a panel of three monoclonal antibodies was used: rabbit monoclonal antihuman ER (clone SP1; Ventana Medical Systems, Inc., Tucson, AZ, USA), directed against the estrogen receptor alpha (ERα) molecule, mouse antihuman directed against the estrogen receptor beta (ERβ, clone EMR02; Novocastra) and rabbit monoclonal antihuman progesterinic receptor (PR, clone 1E2; Ventana, that recognizes the A and B forms of PR receptor). Immunostaining for ERα and PR was performed on 4-μm thick paraffin sections using the BenchMark XT automated staining system (Ventana). For antigen retrieval, slides were heated with Cell Conditioning Solution 1 (CC1) for 30 min.

**Duplicated images in PubPeer**
## Apoptotic phenomena are not a major cause of enteric neuronal loss in constipated patients with dementia

Gabrio Bassotti, Vincenzo Villanacci, Simona Fisogni, Morris Cadei, Francesco Di Fabio and Bruno Salerni

1 Department of Clinical and Experimental Medicine, University of Perugia, Perugia, and 2 2nd Department of Pathology and 3 Surgery, Spedali Civili, Brescia, Italy - Neuropathology 2007; 27, 67–72 doi:10.1111/j.1440-1789.2006.00740.x

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<td>No informed consent or IRB</td>
<td>Patients: Two institutionalized patients (a man, aged 60, and a woman, aged 94) with long-term dementia (defined as a progressive loss of memory and at least one other cognitive function, with sufficient severity to impair normal functioning) and probable diagnosis of Alzheimer’s disease, according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria, were studied.</td>
<td>Controls: Two groups of controls were used: (i) 10 patients (nine women, one man; age range 43–75 years) undergoing left hemicolecction for non-obstructing colorectal cancer, since there is evidence that the distribution of the interstitial cells of Cajal (ICC) is relatively uniform throughout the human colon. 10 No data are available on the regional density of the enteric neurons and glial cells in humans, although in preliminary observations we did not detect significant regional differences between the various colonic segments, except in the rectum (G. Bassotti and V. Villanacci, personal observations, 2006). The control specimens were taken at least 5 cm from the resection margin in tumor free</td>
<td>This paper needs a careful consideration. Indeed, in addition to two very fragile institutionalized patients that were treated without consent, also the controls, i.e. 10 cancer patients and 26 slow transit constipation were treated surgically. No consent is mentioned. Further removing the colon from Alzheimer patients because the constipation is illegal.</td>
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and underwent surgery for the relief of symptoms. No patient had neurovegetative dysfunctions such as urinary incontinence, orthostatic hypotension or neuro-cardiovascular instability. Colonoscopy in both patients only showed the presence of melanosis coli. Anorectal manometry showed normal results in one patient and mild hypotonia of the anal sphincter in the other. No megacolon or megarectum was evident on barium enema. In both patients, subtotal colectomy with ileo-rectal anastomosis was carried out. The clinical follow-up (2 years for the older patient, and 3 years for the other) was unremarkable concerning bowel function, except for a modest increase of the frequency of the evacuations, that at present averages four for both patients constipation unresponsive to any therapeutic mea

areas; and (ii) 26 patients with slow transit constipation (STC, 25 women, one man; age range 24–78 years) undergoing colectomy with ileorectostomy for severe intractable constipation. These patients were part of a study on enteric nervous system abnormalities, and were shown to have a significant decline in enteric nervous structures, particularly gangliar neurons (which also displayed an increased number of apoptotic cells) and ICC, compared to controls. The studies were carried out in accordance with local ethical guidelines, following the recommendations of the Declaration of Helsinki (Edinburgh revision, 2000).