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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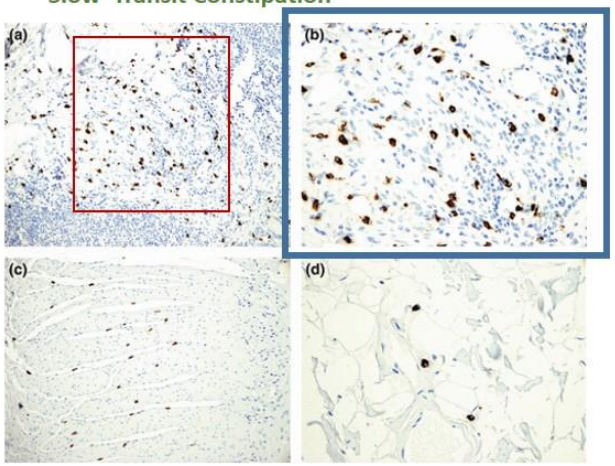
Type study according to the Materials and methods	Patients	Controls	Methods	Comment
Ethical considerations As this was a **retrospective study,** no individual patient identification was involved and no study-driven clinical intervention was performed; therefore no ethical approval was necessary	Archival 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.17, 18 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.19 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
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. Cathomas B. Salerni
First published: 03 May 2011 https://doi.org/10.1111/j.1365-2036.2011.04684.x Citations: 25
Prof. G. Bassotti, Gastroenterology & Hepatology Section,
Department of Clinical and Experimental Medicine, University of Perugia, Piazza Menghini, 1, 06156 San Sisto (Perugia), Italy.

Slow Transit Constipation



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

Diverticular disease

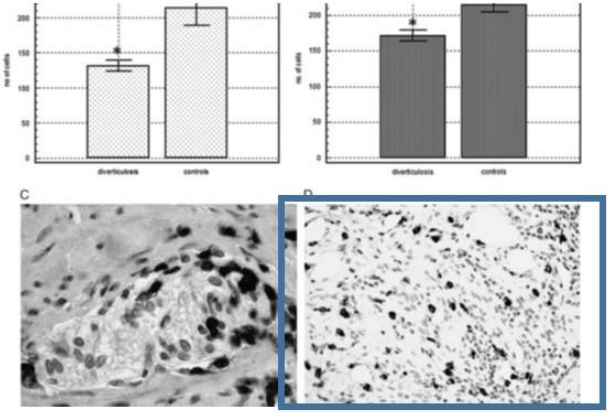


FIGURE 1. A, Histograms showing the decreased number of interstitial cells of Cajal (c-kit assessment by CD117) in patients with diverticulosis compared with controls. *Statistically different from controls. B, Histograms showing the decreased number of enteric glial cells (S-100 assessment) in patients with diverticulosis compared with controls. *Statistically different from controls (adapted from Bassotti et al[15]). C A representative image of lymphocytic infiltration (CD3 assessment) 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.

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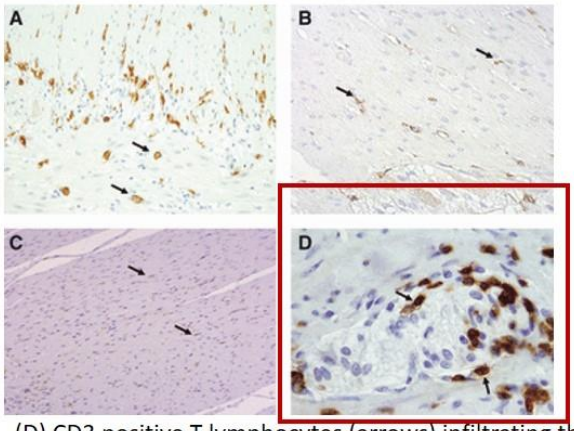
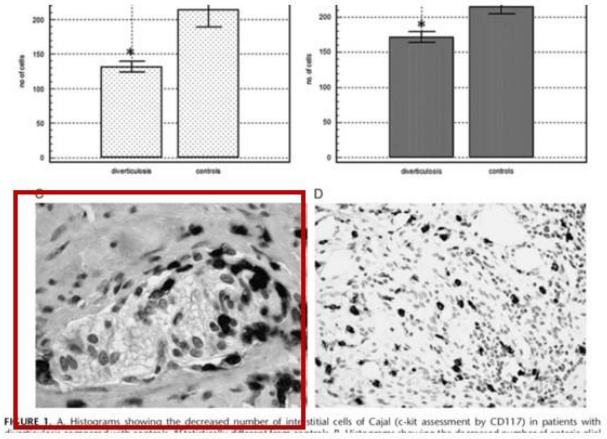


(D) CD3 positive T lymphocytes (arrows) infiltrating the myenteric plexus in a patient with **Crohn's disease**. Original magnification ×1000.



Diverticular disease

DOI:10.1038/labinvest.3700564

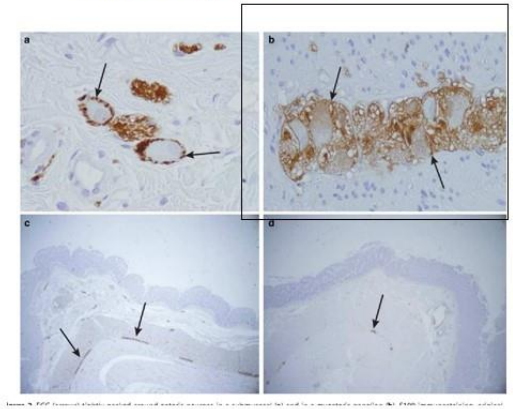


Figure 2 EGC (arrows) tightly packed around enteric neurons in a submucosal (a) and in a myenteric ganglion (b). S100 immunostaining, original magnifications 40 (a) and 100 (b). Full thickness specimen of human colon of a control subject (c) and of a patient with intractable slow-transit constipation (d). The arrows indicate EGC in the myenteric plexus. Note the rarefaction of these cells in (d). S100 immunostaining, original magnification 10.

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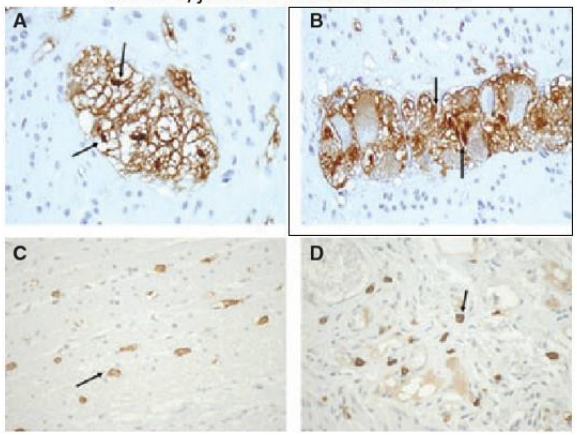

Figure 3 (A) Enteric glial cells (arrows) in the myenteric plexus of a patient with ulcerative colitis, uninvolved area. S100, original magnification ×400. (B) Increased number of enteric glial cells (arrows) in an involved area of the same patient. S100, original magnification ×400. (C) Mast cells (arrows) in the muscularis propria of a patient with ulcerative colitis, involved area. CD 117, 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. CD 117, original magnification ×200.

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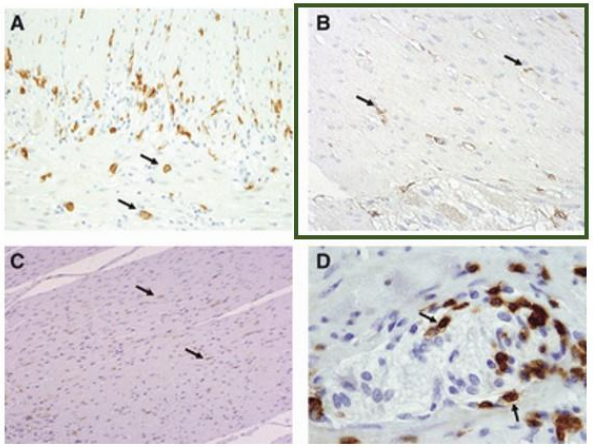


Open in figure viewerPowerPoint
(A) Interstitial cells of Cajal around the myenteric plexus and mast cells in the muscularis propria (arrows) in a **patient with Crohn's disease**. Note the different shape of mast cells with respect to interstitial cells of Cajal. CD 117, original magnification ×200. (B) Intramuscular interstitial cells of Cajal (arrows) in a patient with Crohn's disease. CD 117, original magnification ×200. (C) Intramuscular interstitial cells of Cajal (arrows) in a control. Note that cell bodies are more numerous than that seen in B. CD 117, original magnification ×200. (D) CD3 positive T lymphocytes (arrows) infiltrating the myenteric plexus in a patient with Crohn's disease. Original magnification ×1000.

Gut. 2006 Jan; 55(1): 41–46. doi: 10.1136/gut.2005.073197
The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow trans constipation.
G Bassotti, V Villanacci, C A Maurer, S Fisogni, F Di Fabio, M Cadei, A Morelli, T Panagiotis, G Cathomas and B Salerni

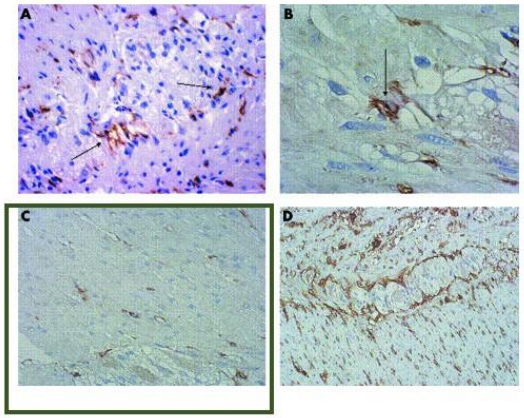
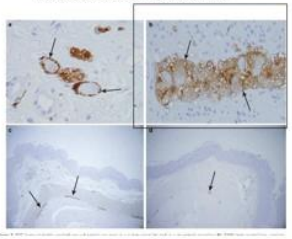


Figure 2 CD117 expression in a control subject (A, B) and in a patient **with slow transit constipation (**C). Note the decrease in interstitial cells of Cajal (ICC) in the patient's tissue. Original magnifications ×4 (A, C) and ×100 (B). ICC are indicated by arrows. (D) CD34 expression in a patient. Original magnificatio

Laboratory Investigation 2012

DOI:10.1038/labinvest.3700564



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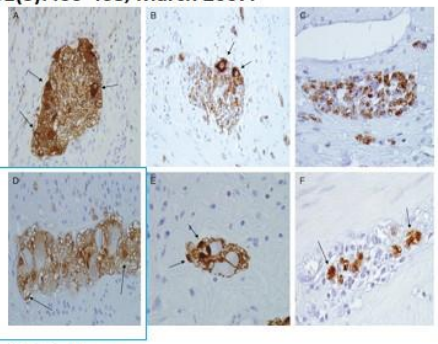
The American Journal of Surgical Pathology 31(3):460-468, March 2007.



Stretched image

Neurogastroenterology and Motility -2008

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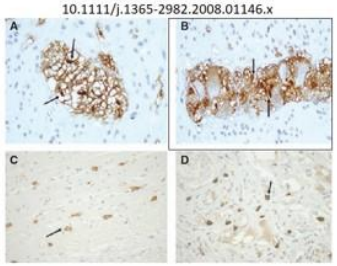

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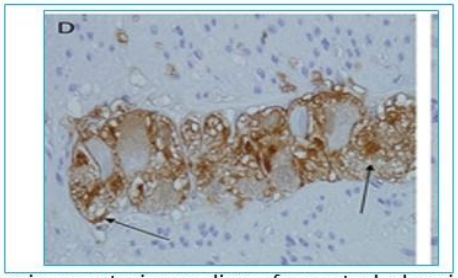


Colonic myenteric ganglion of a control, showing a normal number of glial cells (arrows).

The role of colonic mast cells and myenteric plexitis in patients with diverticular disease

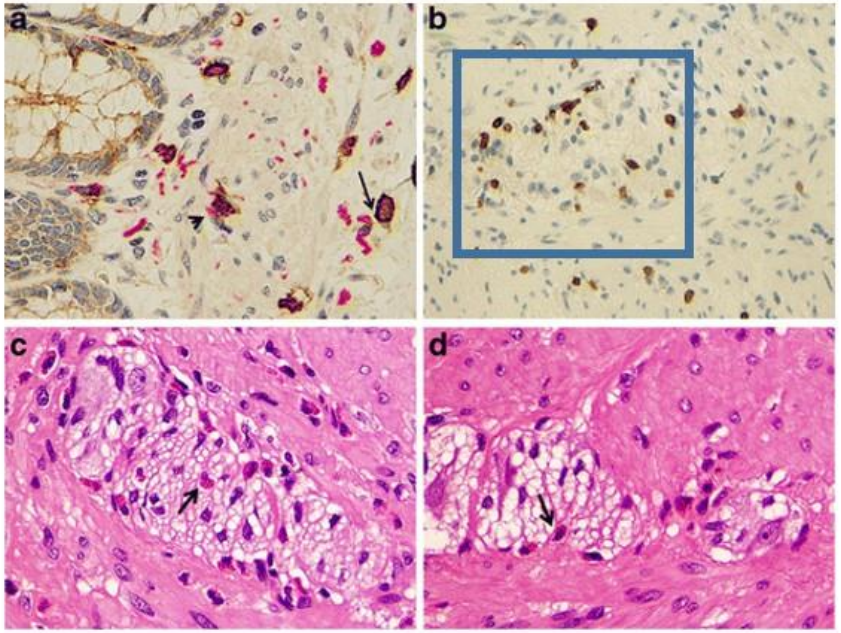
Gabrio Bassotti & Vincenzo Villanacci & Riccardo Nascimbeni & Elisabetta Antonelli & Moris Cadei & Stefania Manenti & Luisa Lorenzi & Amin Titi & Bruno Salerni Accepted: 25 July 2012 / Published online: 5 August 2012

Int J Colorectal Dis (2013) 28:267–272 DOI 10.1007/s00384-012-1554-z

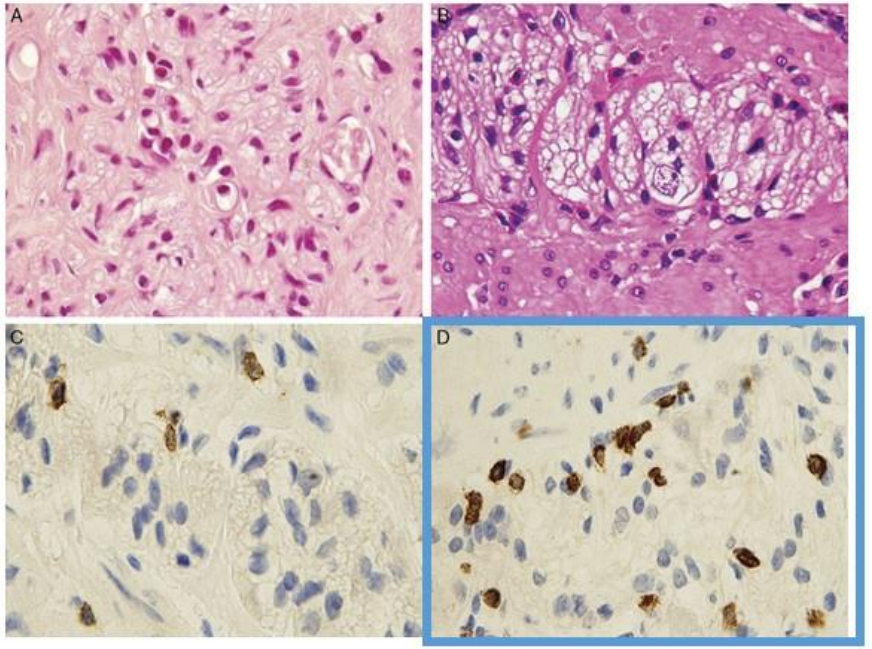
Type study according to the Materials and methods	Patients	Controls	Methods	Comment
Ethical considerations Since this was a retrospective study, no individual patient identification was involved and no study-driven clinical intervention was performed; therefore, no ethical approval was necessary	Full 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	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.	Methods Full-thickness sigmoid samples were obtained from formalinfixed tissue and transversal sections obtained after paraffin embedding were processed for both conventional histology (H&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	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

	[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].		using the monoclonal mouse anti-human CD 3 antibody (Dako Cytomation, dilution 1:40).	
Images in this paper				

Bassotti, G., Villanacci, V., Nascimbeni, R. et al. Int J Colorectal Dis (2013) 28: 267. https://**doi.org/10.1007/s00384-012-1554-z**



Advances In Anatomic Pathology: January 2013 - Volume 20 - Issue 1 - p 17–31 doi: **10.1097/PAP.0b013e31827b65c0**



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

First published:03 May 2011 https://doi.org/10.1111/j.1365-2036.2011.04684.x Citations: 25

Prof. G. Bassotti, Gastroenterology & Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, Piazza Menghini, 1, 06156 San Sisto (Perugia), Italy.
E-mail: gabassot@tin.it

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
Ethical considerationsAs this was a retrospective study, no individual patientidentification was involved and no study-driven clinicalintervention was performed; therefore a simplified IRBapproval was obtained.	PATIENTSSurgical full-thickness specimens from 29 patients (24women, 5 men, age range 27–75 years) undergoing sub-total colectomy with ileorectostomy for severe intractableconstipation were evaluated. Twenty of these patientshave previously been investigated in a study on entericnervous system (ENS) abnormalities.18	As right and left sections of the colon were analysed inpatients, for caecum, ascending and transverse weobtained control samples from 10 patients (eightwomen, two men, age range 38–70 years) undergoingright hemicolectomy and for descending and sigmoidfrom 10 patients (six women, four men, age range 41–78 years) undergoing left hemicolectomy, both for non-obstructing cancer. These patients were not constipatedand 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 takenat least 3 cm from the resection margin in tumour freeareas.	M ETHODS Tissues were fixed in 10% neutral-buffered formalin for24 h, then full-thickness samples from multiple colonicsegments (cecum, ascending, transverse, descending andsigmoid areas) were taken and transversal sectionsobtained. Sections were processed for both conventionalhistology (H&E and Trichrome stain) and immunohisto-chemistry (IHC).MC detectionA specific antibody targeting MC tryptase20(monoclonalmouse clone 10D11, dilution 1:200, Novocastra, UK) wasused. Paraffin sections were dewaxed and rehydratedthrough decreasing alcohol series up to distilled water.The sections were then subjected to heat-induced epitoperetrieval by immersion in a heat-resistant container fille	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 indicatedNOTE this study mention that some of the patients were stuidied in ref. 18. Reference 18 is a paper from GUT. See below

Images in this paper	

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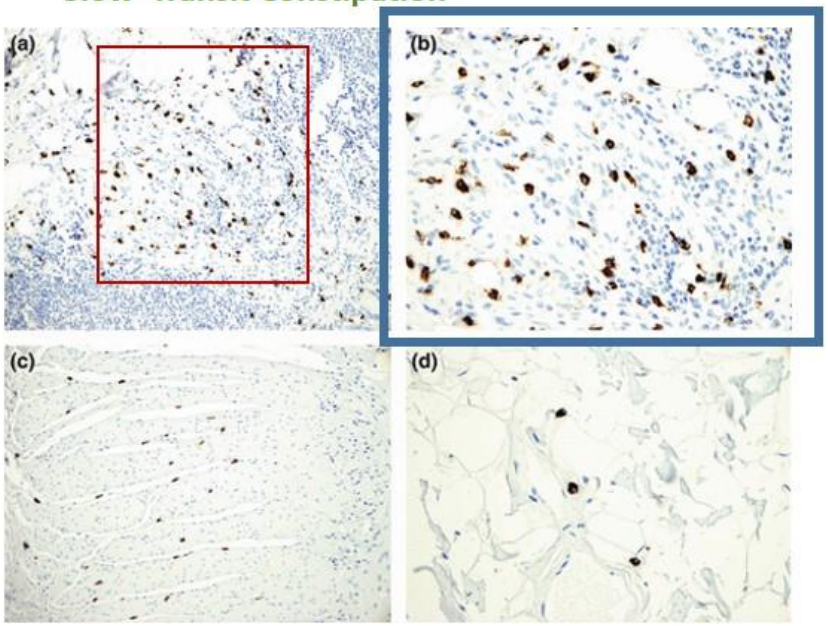
Slow Transit Constipation



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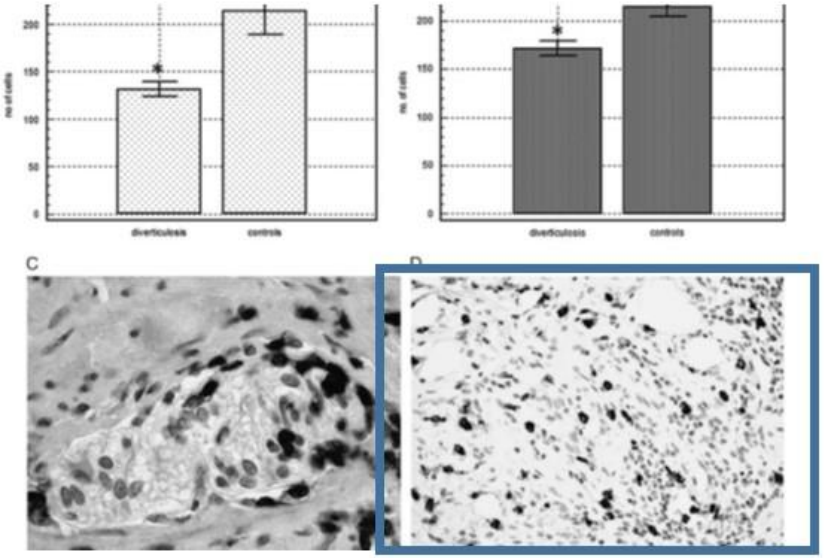
Diverticular disease



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Diverticular Disease of the Colon
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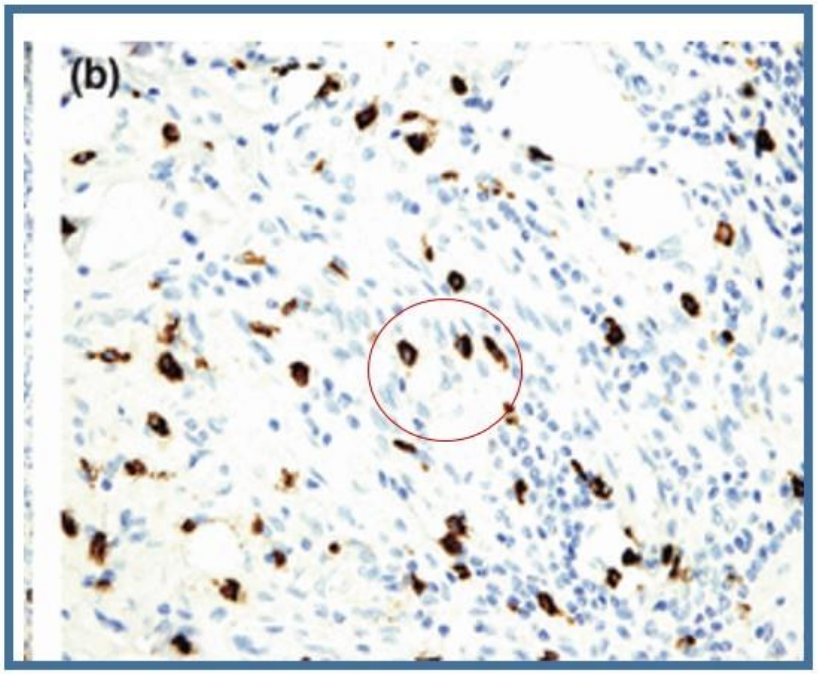
Gabrio Bassotti, MD, PhD,* Vincenzo Villanacci, MD,w
Nunzia Bernardini, MD,z and Maria P. Dore, MD

J Clin Gastroenterol • Volume 50, Supp. 1, October 2016

Representative images of MC in Slow Transit Constipation .



Diverticular disease

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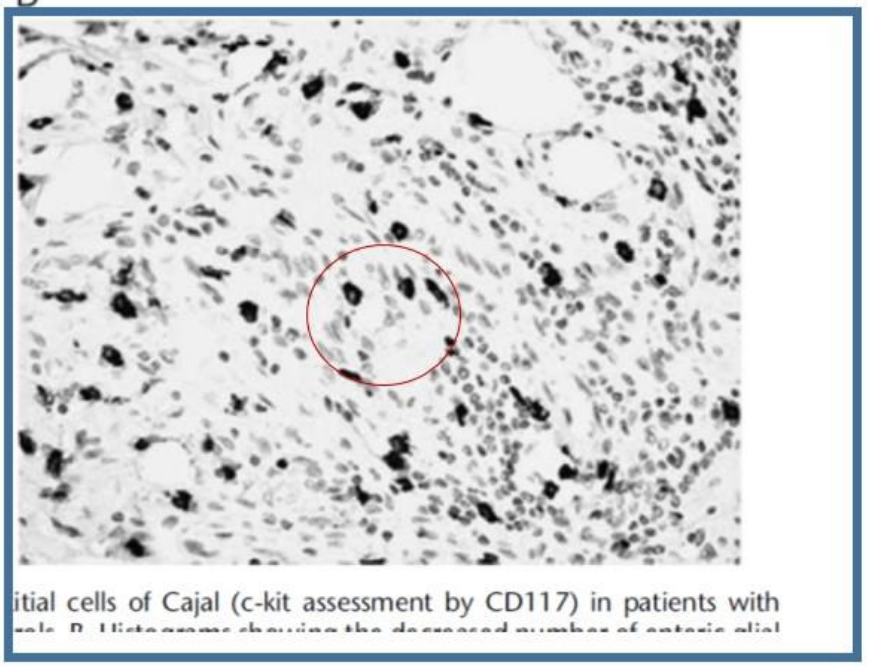

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Gut , 55 (1), 41-6 Jan 2006

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

G Bassotti 1, V Villanacci, C A Maurer, S Fisogni, F Di Fabio, M Cadei, A Morelli, T Panagiotis, G Cathomas, B Salerni

Affiliations expand

PMID: 16041063 PMCID: PMC1856399 DOI: 10.1136/gut.2005.073197

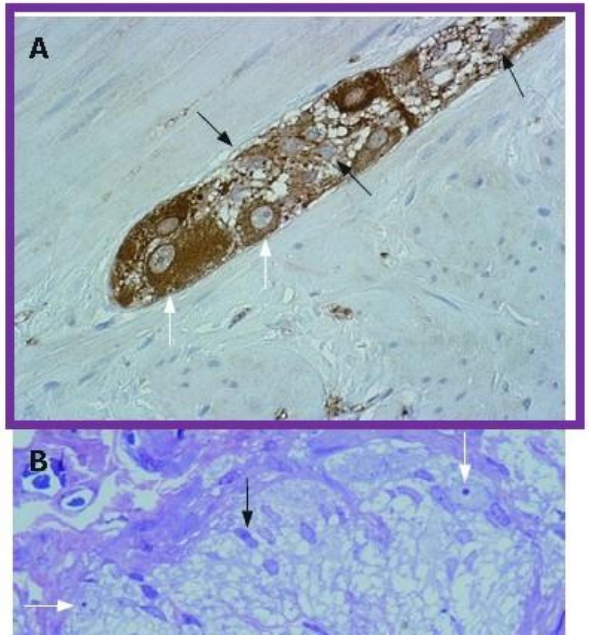
Type study according to the Materials and methods	Patients	Controls	Methods	Comment
No mention to ethic	Twenty six STC patients (25 women, one man; age range 24–78 years) undergoing colectomy with ileorectostomy for severe intractable constipation were enrolled in the study. Inclusion criteria were: (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 hemicolectomy 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.[16] No data are available on the regional density of enteric neurones 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). Control specimens were taken at least 5 cm from the resection margin in tumour free areas.	fter removal, surgical specimens were immediately fixed in 10% neutral buffered formalin for 24 hours, and then 12–20 full thickness samples from the whole esected 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.[20,21] As ICC express Kit,[22] 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.[19] 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.[23] 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.[24] 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	
Images in this paper				

World J Gastroenterol. 2007 Aug 14; 13(30): 4035–4041.
Published online 2007 Aug 14. doi: **10.3748/wjg.v13.i30.4035**

Enteric glial cells and their role in gastrointestinal motor abnormalities: Introducing the neuro-gliopathies

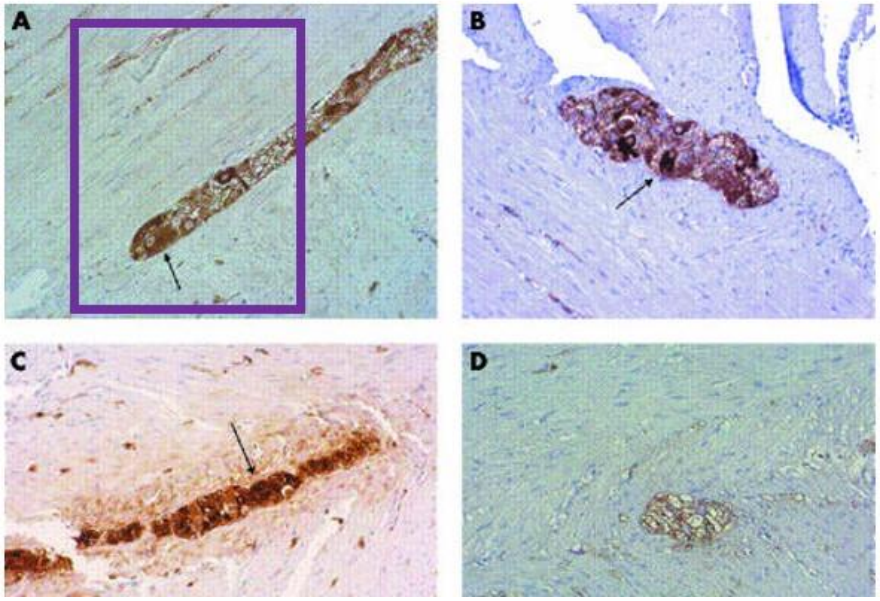
Gabrio Bassotti, Vincenzo Villanacci, Simona Fisogni, Elisa Rossi, Paola Baronio, Carlo Clerici, Christoph A Maurer, Gieri Cathomas, and Elisabetta Antonelli



Gut. 2006 Jan; 55(1): 41–46.
doi: **10.1136/gut.2005.073197**

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, and B Salerni



The enteric nervous system in patients with calculous and acalculous gallbladder

VincenzoVillanacci aRacheleDel Sordo Marianna Salemme Moris Cadei Angelo Sidoni GabrioBassotti
Show more

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

Type study according to the Materials and methods	Patients	Controls	Methods	Comment

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	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				

Techniques in Coloproctology
November 2018, Volume 22, Issue 11, pp 857–866| Cite as

Optimal processing of ESD specimens to avoid pathological artifacts
L. Reggiani BonettiEmail authorR. MantaM. MannoR. ConigliaroG. MissaleG. BassottiV. Villanacci
Techniques in Coloproctology (2018) 22:857–866 https://doi.org/10.1007/s10151-018-1887-x

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
. Ethical issues Since this was a retrospective study with anonymously collected data, and without other interventions, no ethical approval from the Ethics Committee was required.	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 orLGI tract ESD after the endoscopic detection of mucosal lesions, endoscopically classified according to the Paris criteria [8].		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.	THIS PAPER IS A FAKE. THE BIOPTIC ISTRUMENTS WAS DESCRIBED FIRST BY THESE AUTHORS IN 2012 REFERENCE 2 AND 7.. SO IT IS IMPOSSIBLE TO HAVE USED IT FOR SAMPLES COLLECTED IN 2000-2205!
Images in this paper Check PubPeer				

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Pathology (August 2011) 43(5), pp. 465–471
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§

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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.	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		The primary endpoint of the study was the number of Foxp3þ cells per field as measured by immunohistochemistry. Further immunohistochemical assessments comprised the count of CD3, CD4, and CD8 T cells. The proportion of Foxp3þ 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 Foxp3þ 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	THIS Study describes the uses of biospies from 216 without consent. The Instituttional review board permit is not described (date and number). The informed cosnent WAS considered requesrted. However, histology sections were cutted ex-novo from stored sampels for CD_3 and $FoxP_3$ immunohistochemistry

	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 (n¼2), stage II (n¼6), stage III (n¼13)]. 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 ¼ 5), stage II (n ¼ 15), stage III (n ¼ 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 988C 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.			
Images in this paper Check PubPeer				

Enteric neuroglial apoptosis in inflammatory bowel diseases
Gabrio Bassotti, Vincenzo Villanacci, Riccardo Nascimbeni, Moris Cadei, Simona Fisogni, Elisabetta Antonelli, Nadia Corazzi, Bruno Salerni
Journal of Crohn's and Colitis, Volume 3, Issue 4, December 2009, Pages 264–270,
https://doi.org/10.1016/j.crohns.2009.06.004

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
3.4 Ethical considerations Since 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.	**2 Patients and methods** 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.	Control specimens were obtained from patients undergoing colonic (n = 10, 5 man 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	**3.1 Immunohistochemistry** 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.[17–20] Apoptosis was evaluated with two methods: by evaluating the expression the caspase-3 (a so-called executioner caspase),[22] and by a monoclonal antibody to single-stranded DNA, using the formamide monoclonal antibody (formamide-MAb) method,[23] as previously described.[17–20] NSE and S-100 immunostaining was carried out, as previously	Again this paper uses surgical samples from patients with colitis and control subjects (colon cancer patients) without patient consent and authorization by etical 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 .	
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Interstitial cells of Cajal, enteric nerves, and glial cells in colonic diverticular disease

Bassotti G, Battaglia E, Bellone G, Dughera L, Fisogni S, Zambelli C, Morelli A, Mioli P, Emanuelli G, Villanacci V. Interstitial cells of Cajal, enteric nerves, and glial cells in colonic diverticular disease. J Clin Pathol. 2005 Sep;58(9):973-7. doi: 10.1136/jcp.2005.026112. PMID: 16126881; PMCID: PMC1770814.

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
The studies were carried out in accordance with local ethical guidelines, following the recommendations of the Declaration of Helsinki (Edinburgh revision, 2000	Patients Colon specimens were obtained from 39 patients (17 men, 22 women; age range, 49–78 years) undergoing elective left hemicolectomy for diverticular disease (35 cases) or emergency surgery for acute diverticulitis with pericolic 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.).	Controls We used 10 specimens from age and sex matched subjects undergoing left hemicolectomy 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,[21] we investigated PGP 9.5 (protein gene product 9.5), a cytoplasmic protein that acts as a marker of general neural tissue,[22,23] and the glial marker protein S-100.[24] Ganglion cells were assessed with a monoclonal anti-PGP 9.5 antibody (IgG2a; 1/100 dilution; Biomeda, Foster	Agaion this paper reports on the use of surgical samples from patients with diverticuklar disease and control subjects (colon cancer) with no patients consnet

			City, California, USA); 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. .	
Images in this paper Check PubPeer				

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

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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.	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 constipation35 (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	Controls 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	. 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	This paper uses again surgical samples for ex novo studies without informed consent from patients and control (colon cancer patients).

	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 disorders36 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 (Biomeda, Foster City, CA, USA).	
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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

Journal of Crohn's and Colitis, Volume 10, Issue 10, October 2016, Pages 1194–1204, https://doi.org/10.1093/ecco-jcc/jjw076

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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	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		2.2.2. Histochemistry Tissue collagen and elastic fibre deposition were evaluated by Sirius Red/Fast Green and orcein, as previously reported. 19 , 2	This paper report the acquisition of IRB but the the institution that released the IRB is not reported. Further, authors are from several instituitions. Additionally, the Oviedo Convention clearly states that the patients consent can NOT be omitted and IRB is NOT a substitute for the informed consent.

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Eosinophilia – associated basal plasmacytosis: an early and sensitive histologic feature of inflammatory bowel disease
Gabriella Canavese Vincenzo Villanacci Elisabetta Antonelli Moris Cadei Anna Sapino Rodolfo Rocca Marco Daperno Renzo Suriani Maria Giulia Di santo Paola Cassoni … See all authors
First published:25 January 2017 https://doi.org/10.1111/apm.12639 Citations: 7

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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.	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		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 MI15 – 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.			
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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 CathomasFirst Published December 18, 2014 Research Article Find in PubMed
https://doi.org/10.1177/2050640614563822

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
Ethical considerations Because 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.	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 classification24 and patients undergoing elective surgery after either the third or fourth attack of diverticulitis25 or for sigmoid stenosis, due to recurrent episodes of diverticulitis.26	We obtained control samples from the proximal resection margin of 15 patients (seven women and eight men; age range 44–83 years) undergoing left hemicolectomy 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.	Methods Archival resection samples from the proximal resection margins of patients and controls were always analyzed according to a standard protocol. The proximal resection margin was chosen, in order to have a homogeneous standard and to avoid architectural distortions, due to the inflammatory process of diverticulitis. We obtained	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 workoput for diverticular disease or colon cancer

			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.	
Images in this paper Check PubPeer				

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 1

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

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
There is no mention to any etrhical consideration or consent	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).	.	Immunohistochemistry EGFR (HER1) receptor status was analyzed by the EGFR pharmDx kit (DAKOCytomation, Carpinteria, CA, USA). FIuorescence 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	This paper uses surgical samples from 64 cancers patients for ex novo studies without informed consent. IRB is not mentioned

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Expression of the Rai (Shc C) adaptor protein in the human enteric nervous system Neurogastroenterol Motil (2008) 20, 206–212 doi: 10.1111/j.1365-2982.2007.01017.x				
Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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	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.	.	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	This paper uses again surgical samples for ex novo studies without informed consent from patients. Seedveral center were involved. Brescia, Milan, Perugia, Liestal (Swisserland). The institution that released the IRB is not mentioned. Animal etical committee is not described. Myenteric Myenteric

			(mouse monoclonal antibody; Novocastra Laboratories, Newcastle upon Tyne, UK, dilution 1 : 100). Sections were evaluated by means of a Olympus BX 41 (Olympus Optical Ltd, Tokyo, Japan) microscope equipped with a Olympus DP 70 digital camera.	
Images in this paper Check PubPeer				

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 ...

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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				Pub Peer and retraction watch.
Images in this paper Check PubPeer				

Is pseudomelanosis coli a marker of colonic neuropathy in severely constipated patients? Villanacci V, Bassotti G, Cathomas G, Maurer CA, Di Fabio F, Fisogni S, Cadei M, Mazzocchi A, Salerni B. Is pseudomelanosis coli a marker of colonic neuropathy in severely constipated patients? Histopathology. 2006 Aug;49(2):132-7. PubMed PMID: 16879390				
Type study according to the Materials and methods	Patients	Controls	Methods	Comment
The studies were carried out in accordance with local ethical guidelines, following the recommendations of the Declaration of Helsinki (Edinburgh revision, 2000).	Patients and methods Specimens were examined from 16 female patients (age range 24–77 years) fulfilling the Rome II criteria for constipation18 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	.	lmunohistochemistry 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	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 idication for colon resection. Why the informed consent for histology stsudies was not

	patients was thought to be due to the ingestion of anthraquinone laxatives, since all of them had used such compounds (mostly the senna-containing overthe-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.19 Histological and immunohistochemical evaluations were carried out according to previously described techniques.19,20 After removal, the surgical specimens were immediately fixed in 10% neutral-buffered formalin for 24 h, aft		histology were indeed macrophages (Figures 2A).	requeted? Multiple center involved : brescia , Perugia and Liestal. There is concern over the indication for surgery.
Images in this paper				

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Type study according to the Materials and methods	Patients	Controls	Methods	Comment
	.26	.	▪	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 workoput for diverticular disease or colon cancer
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An assessment of enteric nervous system and estroprogestinic receptors in obstructed defecation associated with rectal intussusception
Neurogastroenterol Motil (2012) 24, e155–e161
10.1111/j.1365-2982.2011.01850.x
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*Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, Perugia,
Italy

Type study according to the Materials and methods	Patients	Controls	Methods	Comment
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.	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	**Controls** 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.	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-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.	Surgical samples for ex novo studies without informed consent from patients (constipation) and control (rectal cancer patients).

			The NSE and S-100 immunostaining was carried out using a peroxidase-based visualization kit (Dako LSAB_), following the manufacturer's ecommendations. 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 heatinduced 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 (ERa) molecule, mouse antihuman directed against the estrogen receptor beta (ERb, clone EMR02; Novocastra) and rabbit monoclonal antihuman progestinic receptor (PR, clone 1E2; Ventana, that recognizes the A and B forms of PR receptor). Immunostaining for ERa and PR was performed on 4-lm 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..	
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Apoptotic phenomena are not a major cause of enteric neuronal loss in constipated patients with dementia

Gabrio Bassotti,1 Vincenzo Villanacci,2 Simona Fisogni,2 Morris Cadei,2 Francesco Di Fabio3 and Bruno Salerni3 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

	Patients	Controls	Methods	Comment
No informed consent or IRB	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.6–9 Both patients came to our attention because of severe ures (including high fiber diet, stimulant and osmotic laxatives, and enemas),	Controls Two groups of controls were used: (i) 10 patients (nine women, one man; age range 43–75 years) undergoing left hemicolectomy 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		This paper needs a carefull consideration. Indeed, in addition to two very fragile intitutionalized 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.

	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 patientsconstipation 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.4 The studies were carried out in accordance with local ethical guidelines, following the recommendations of the Declaration of Helsinki (Edinburgh revision, 2000).		