

## What's New in Diagnosing Diverticular Disease

Davor Štimac<sup>1</sup>, Gerardo Nardone<sup>2</sup>, Andrea Mazzari<sup>3</sup>, Antonio Crucitti<sup>3</sup>, Giovanni Maconi<sup>4</sup>, Walter Elisei<sup>5</sup>, Alessandra Violi<sup>6</sup>, Antonio Tursi<sup>7</sup>, Francesco Di Mario<sup>6</sup>

- 
- 1) Department of Gastroenterology, Clinical Hospital Centre Rijeka, Faculty of Medicine, University of Rijeka, Rijeka, Croatia  
2) Gastroenterology Unit, Department of Clinical Medicine & Surgery University Federico II of Naples, Italy  
3) General Mininvasive Surgery Cristo Re Hospital, Catholic University Rome  
4) Gastrointestinal Unit, Department of Clinical Sciences, "L.Sacco" University Hospital, Milan, Italy  
5) Unit of Gastroenterology and Endoscopy - San Camillo Forlanini Hospital, Rome, Italy  
6) Gastrointestinal Unit, Department of Medical and Surgical Sciences, "Maggiore" University Hospital, Parma, Italy  
7) Territorial Gastroenterology Service, ASL BAT, Andria, Italy

*Address for correspondence:*  
**Francesco Di Mario**, MD  
Gastrointestinal Unit,  
Department of Medical and Surgical Sciences  
"Maggiore" University Hospital  
43121 Parma, Italy  
[francesco.dimario@unipr.it](mailto:francesco.dimario@unipr.it)

### ABSTRACT

In this session different issues for the diagnosis of diverticular disease (DD) were considered including "Biomarkers", "Computer tomography", "Ultrasonography in detecting acute diverticulitis", "Endoscopy" and "The DICA classification: a new predictive tool in managing diverticular disease". Most patients affected by DD suffer from recurrent attacks of abdominal pain without evidence of an active inflammatory process, causing a difficult differential diagnosis with other intestinal conditions. Several biomarkers, serological, fecal, urinary and genetic were considered, but recent studies confirmed that only CRP and fecal calprotectin are matching with the criteria for an ideal biomarker for DD. Colonoscopy still remains the gold standard for the diagnosis of DD, playing a key role in many clinical settings, such as colonic diverticular bleeding, or to differentiate inflammatory bowel disease (IBD) and segmental colitis associated with diverticulosis (SCAD); Moreover, in 2015 has been developed the DICA (Diverticular Inflammation and Complication Assessment) endoscopic classification that considers 10 different parameters, each one with a score, and the sum of items scores represents the severity of the disease; in this way the endoscopic exam would be able to predict the outcome of DD for each patient. On the other hand, computer tomography (CT) is the gold standard for acute diverticulitis (AD) with an excellent sensitivity and specificity; recently, metanalysis of prospective studies have shown that intestinal ultrasonography (IUS) and CT have the same sensitivity for the diagnosis of an AD and the advantage is that IUS is less expensive, non-invasive and easily accessible.

**Key words:** diverticular disease – acute diverticulitis – colonoscopy – DICA classification – computer tomography – ultrasonography – fecal calprotectin.

**Abbreviations:** AD: acute diverticulitis; CRP: C reactive protein; CT: computer tomography; CTC: computer tomographic colonography; DD: diverticular disease; DICA: Diverticular Inflammation and Complications Assessment; ESR: erythrocyte sedimentation rate; FC: fecal calprotectin; IBD: inflammatory bowel diseases; IUS: intestinal ultrasonography; LGIB: lower gastrointestinal bleeding; SCAD: segmental colitis associated with diverticulosis.

### BIOMARKERS IN THE DIAGNOSIS OF COLONIC DIVERTICULAR DISEASE

Clinical evaluation alone of DD results in a wrong diagnosis in 34-68% of the cases and may lead to inadequate treatment, unneeded investigations, unnecessary hospital stay and increased costs. The use of imaging techniques may help clinicians. However, ultrasound is examiner-dependent and

(CT) is expensive and potentially harmful. Biomarkers are measurable indicators of some biological conditions and may allow the characterization of disease subtypes. Ideal biomarkers should be, accurate, reproducible, non-invasive and low cost. Nowadays, serum, fecal, urinary and genetic biomarkers have been proposed for DD.

#### Serum biomarkers

C reactive protein (CRP), erythrocytes sedimentation rate (ESR), white blood cell counts (WBC), fibrinogen,  $\beta$ -2-globulin,  $\alpha$ 1-acid glycoprotein were increased in patients with AD. However, by a multivariate analysis only CRP >50 mg/dl was an independent predictor of AD [1]. Further, CRP > 150 mg/dl significantly discriminated uncomplicated diverticulitis from complicated diverticulitis [2]. High levels of serum procalcitonin, a marker of bacterial infection, differentiated (sensitivity 80%

specificity 91%) complicated versus uncomplicated diverticulitis when combined with CT scans [3].

#### **Fecal biomarkers**

When there is inflammation in the gastrointestinal tract, calprotectin is delivered by neutrophils in the stool. High levels of fecal calprotectin (FC) may differentiate IBS from IBD, thus it is a sensitive marker of activity in IBD. FC correlates with mucosal leucocytes density and may distinguish symptomatic DD from IBS [4]. Further, FC may be useful in assessing response to therapy and in predicting diverticulitis recurrence [5]. Microbiota imbalance is a risk factor for the occurrence of DD. Patients with DD showed higher amount of Enterobacteriaceae [6], a depletion of microbiota members with anti-inflammatory activity and metabolome profiles linked with inflammatory pathways and gut neuromotor dysfunction [7].

#### **Urinary biomarkers**

Because of dysbiosis, it is hypothesized that specific urinary metabolic pathways might identify patients with DD. Hippurate and methanol showed significant differences among health controls and patients with AD and symptomatic uncomplicated DD (SUDD) [8].

#### **Genetic markers**

Genome wide studies found associations between specific loci and DD (ARHGAP15 and COLQ) and AD (FAM155A and rs9960286 located near CTAGE1) [9,10].

### **ENDOSCOPY**

Colonoscopy plays a key role in different clinical settings of DD: 1) diverticular bleeding; 2) differential diagnosis of colon diseases (SCAD vs IBD); 3) follow-up AD; 4) prognostic tool in patients with DD.

#### **Colonoscopy in Colonic Diverticular Bleeding**

Colonic Diverticular Bleeding is the most common cause of lower gastrointestinal bleeding (LGIB) affecting from 3 to 15% of patients with colonic diverticulosis, with mortality rate from 2 to 3 %. In patients with suspected colonic diverticular bleeding colonoscopy is generally indicated: a) electively when bleeding has stopped spontaneously (70-80% of cases): in order to exclude other causes of LGIB. b) as primary intervention in managing colonic diverticular bleeding: urgent colonoscopy, within 24 hours, in order to find signs of diverticular bleeding (active bleeding, visible vessel or adherent clot). c) as primary imaging in patients with recurrent episodes of LGIB in which CT angiography was non-diagnostic [11,12,13]. Unprepared colonoscopy is not recommended because is associated with a low cecal intubation rate (55-70%) and a high risk of bowel perforation. Urgent colonoscopy for acute LGIB is associated with a shorter length of hospital stay and lower hospitalization costs [14,15,16].

#### **Colonoscopy in differential diagnosis of colon diseases (SCAD vs IBD)**

Segmental colitis associated with diverticulosis (SCAD) is a chronic inflammatory process localized in the interdiverticular

mucosa and therefore mainly in the sigmoid colon. By definition, the diverticular ostia are spared from any inflammation [17]. There are histological similarities between SCAD and IBD, but by endoscopic examination we can easily differentiate SCAD from IBD or other type of colitis: in SCAD the inflammatory process involves the interdiverticular mucosa in the colonic area presenting diverticulosis and therefore mainly in the sigmoid colon, and the rectum and proximal colon are endoscopically and histologically normal; in ulcerative colitis the rectum is always affected; Crohn's disease may affect colon and other gastrointestinal areas [18].

#### **Colonoscopy following Acute Diverticulitis**

Regarding the role of endoscopy in AD or following an attack of acute diverticulitis, Galetin and colleagues [19], in a recent systematic review and comparison of guidelines, confirm that there is discordance in performing colonoscopy in acute diverticulitis. Colonoscopy is usually avoided in patients with suspicion of AD because of the high risk of bowel perforation. Expert opinion is in favour of performing these tests when the acute process has resolved, usually after approximately 6 weeks, to rule out the presence of other diseases, such as cancer and IBD. Colonoscopy following AD is useful in the following conditions: a) In persistent symptomatic patients in order to exclude other diseases b) After resolution of an AD if a high-quality examination of the colon has not been recently performed [20].

#### **Colonoscopy as a predictive tool for diverticular disease outcomes**

Diverticular Inflammation and Complication Assessment (DICA) is an endoscopic classification that considers four items of DD in a scoring system and is a promising tool for DD outcomes.

### **THE DICA CLASSIFICATION: A NEW PREDICTIVE TOOL IN MANAGING DIVERTICULAR DISEASE**

Diverticulosis of the colon is the most frequent anatomic alteration detected during screening colonoscopy [21]. It can be detected in 32.6% of routine colonoscopies and up to 71.4% of people  $\geq 80$  years. [21]. Moreover, the SUDD patients having extensive diverticulosis are at higher risk of AD occurrence [22], and the persistence of endoscopic inflammation may be a risk factor for AD recurrence [23]. Despite these data, an endoscopic classification of diverticulosis and DD was absent till 2015. Recently the first endoscopic classification of diverticulosis and DD, called DICA has been developed and validated. This classification takes into account four main items and several subitems: the extension of diverticulosis (left or right), the number of diverticula per each colonic region ( $\leq 15$  or  $\geq 15$  diverticula), the presence of inflammation (oedema, hyperaemia, erosions, SCAD), the presence of complications (rigidity, stenosis, pus and bleeding). Each of these items and subitems has a numerical score, and the sum of the scores lead to three different DICA scores: DICA 1 (up to 3 points), DICA 2 (from 4 to 7 points), and DICA 3 (over 7 points) [24]. This classification seems to have a predictive value on the

outcome of DD in terms of AD occurrence/recurrence and risk of surgery, founding that DICA 3 patients were at higher risk of AD occurrence/recurrence compared with DICA 2 or DICA 1 patients. The same risk was recognized in assessing the surgical risk: DICA 3 patients were at higher risk of surgery linked to the disease than DICA 2 or DICA 1 patients [25]. A recent study in real life confirms the significant agreement for this classification in clinical settings, even for endoscopists not expert with this disease [26]. DICA classification has become the standard reference for the studies assessing DD by an endoscopic point of view [27-29]. A prospective, international study is currently ongoing. This study will take three years, and the results at one year of follow-up have recently become available: the preliminary analysis seems to confirm the results of the retrospective study, namely DICA 3 patients are at higher risk of AD and surgical procedures disease-related than DICA 2 and DICA 1 patients [30].

## COMPUTER TOMOGRAPHY

Computer tomography (CT) is the gold standard in diagnosing and staging patients with AD. CT with i.v. contrast performed within 48 hours after onset of symptoms has excellent sensitivity and specificity (98% and 99%). It is useful for diagnosis, treatment and follow up of patients. There are several CT findings: maximal thickness of the bowel, inflammation of pericolic fat, presence of abscesses, stenosis, fistula, free air and fluid in the peritoneal cavity. It's also able to identify the length of colonic inflammation. CT scan grades the severity of diverticulitis; several CT classifications have been proposed: Ambrosetti, Hynchey, WSES. Currently the modified Hynchey's classification remains the most widely used, but it is insufficient to cover all clinical presentations [31-33]. CT scan should be descriptive taking into consideration the details of all the signs that might play a role in the evaluation of AD. CT is a significant predictor of surgery during the first attack, the presence of extraintestinal gas  $\geq 5$  mm being correlated with unfavourable outcome of nonsurgical treatment [34]. Length of involved colon  $> 5$  cm and retroperitoneal abscess were associated with diverticulitis recurrence: distant intraperitoneal air is the most important factor predicting surgical treatment [35, 36]. CT colonography (CTC) recently has been proposed as a diagnostic test in patients recovering from an episode of AD; CTC should be performed at least 2 or 3 months after the acute episode of diverticulitis. A DD severity score based on CTC findings has been proposed. The central place of CT in the evaluation of AD severity is proven. A classification system based on CT scan results may drive decisions making [37].

## ULTRASONOGRAPHY

Several international guidelines considered intestinal ultrasonography (IUS) the first imaging technique for detecting AD [38]. Meta-analysis of prospective studies have shown that IUS and CT scan have the same sensitivity in diagnosing AD, and both techniques can be used as initial diagnostic tool. However, CT has the advantage of a more panoramic view and it is likely more useful to identify alternative diseases [39]. On the other hand, US is widely available and easily

accessible within the emergency department, it has a low cost, it's noninvasive and it can be therefore performed as first exam in patients with abdominal pain, followed by CT scan in inconclusive cases. Another main advantage of IUS is its ability to assess in real-time the site of abdomen with the greatest tenderness, in cases with localized and well-defined abdominal pain. In patients with uncomplicated AD, at the level of areas with maximum tenderness, IUS can detect short-segmental bowel wall thickening ( $> 5$  mm), an inflamed diverticulum, and localized hypertrophy of mesenteric fat. The presence of at least 2 of these signs allows the diagnosis of AD with a sensitivity and specificity greater than 90%. In patients with AD complicated by fistulas or abscesses, pericolic hypoechoic or anechoic structures may be observed within the mesenteric fat hypertrophy. Differentiation between phlegmonous and septic fluid collections may be obtained by using color Doppler or contrast-enhanced US, which are able to detect the hypervascularization of the inflammatory areas [40]. A potential limitation of IUS might be the need of an expert sonographer. This issue, namely level of experience of an operator in detecting AD has been assessed in a Dutch study, showing that sensitivity of a radiologists with experience of  $< 500$  intestinal exams is only 58% compared with 82% of an expert one. However, the positive predictive values were similar 90% for expert vs. 85% for non-expert radiologists [41]. Anyway, it should be recognized that every diagnostic technique requires experience, and hopefully in future the learning of intestinal US in medical schools will overcome this limitation.

## REFERENCES

1. Andeweg CS, Knobben L, Hendriks JC, Bleichrodt RP, van Goor H. How to diagnose acute left-sided colonic diverticulitis. Proposal for a clinical scoring system. *Ann Surg* 2011;253:940-946. doi:10.1097/SLA.0b013e3182113614
2. Mäkelä JM, Klintrup K, Takala H, Rautio T. The role of C-reactive protein in prediction of the severity of acute diverticulitis in an emergency unit. *Scand J Gastroenterol* 2015;50:536-541. doi:10.3109/00365521.2014.999350
3. Jeger V, Pop R, Forudastan F, Barras JP, Zuber M, Piso RJ. Is there a role for procalcitonin in differentiating uncomplicated and complicated diverticulitis in order to reduce antibiotic therapy? A prospective diagnostic cohort study. *Swiss Med Wkly* 2017;147:w14555. doi:10.4414/smw.2017.14555
4. Tursi A, Brandimarte G, Elisei W, Giorgetti GM, Inchingolo CD, Aiello F. Faecal calprotectin in colonic diverticular disease: a case-control study. *Int J Colorectal Dis* 2009;24:49-55. doi:10.1007/s00384-008-0595-9
5. Tursi A, Elisei W, Picchio M, Brandimarte G. Increased faecal calprotectin predicts recurrence of colonic diverticulitis. *Int J Colorectal Dis* 2014;29:931-935. doi:10.1007/s00384-014-1884-0
6. Linninge C, Roth B, Erlanson-Albertsson C, Molin G, Toth E, Ohlsson B. Abundance of Enterobacteriaceae in the colon mucosa in diverticular disease. *World J Gastrointest Pathophysiol* 2018;9:18-27. doi:10.4291/wjgp.v9.i1.18
7. Barbara G, Scaiola E, Barbaro MR, et al. Gut microbiota, metabolome and immune signatures in patients with uncomplicated diverticular disease. *Gut* 2017;66:1252-1261. doi:10.1136/gutjnl-2016-312377

8. Tursi A, Mastromarino P, Capobianco D, et al. Urinary metabolic profiling and symptomatic uncomplicated diverticular disease of the colon. *Clin Res Hepatol Gastroenterol* 2017;41:344-346. doi:10.1016/j.clinre.2017.01.006
9. Schafmayer C, Harrison CW, Buch S, et al. Genome-wide association analysis of diverticular disease points towards neuromuscular, connective tissue and epithelial pathomechanisms. *Gut* 2019;68:854-865. doi:10.1136/gutjnl-2018-317619
10. Sigurdsson S, Alexandersson KE, Sulem P, et al. Sequence variants in ARHGAP15, COLQ and FAM155A associate with diverticular disease and diverticulitis. *Nat Commun* 2017;8:15789. doi:10.1038/ncomms15789
11. Suzuki K, Uchiyama S, Imajyo K, et al. Risk factors for colonic diverticular hemorrhage: Japanese multicenter study. *Digestion* 2012;85:261-265. doi:10.1159/000336351
12. Nagata N, Niikura R, Aoki T, et al. Colonic diverticular hemorrhage associated with the use of nonsteroidal anti-inflammatory drugs, low-dose aspirin, antiplatelet drugs, and dual therapy. *J Gastroenterol Hepatol* 2014;19:1786-1793. doi:10.1111/jgh.12595
13. Longstreth GF. Epidemiology and outcome of patients hospitalized with acute lower gastrointestinal hemorrhage: a population-based study. *Am J Gastroenterol* 1997;92:419-424.
14. Yen EF, Ladabaum U, Muthusamy VR, Cello JP, McQuaid KR, Shah JN. Colonoscopic treatment of acute diverticular hemorrhage using endoclips. *Dig Dis Sci* 2008;53:2480-2485. doi:10.1007/s10620-007-0151-4
15. Kaltenbach T, Watson R, Shah J, et al. Colonoscopy with clipping is useful in the diagnosis and treatment of diverticular bleeding. *Clin Gastroenterol Hepatol* 2012;10:131-137. doi:10.1016/j.cgh.2011.10.029
16. Wong Kee Song LM, Baron TH. Endoscopic management of acute lower gastrointestinal bleeding. *Am J Gastroenterol* 2008;103:1881-1887.
17. Koutroubakis IE, Antoniou P, Tzardi M, Kouroumalis EA. The spectrum of segmental colitis associated with diverticulosis. *Int J Colorectal Dis* 2005;20:28-32. doi:10.1007/s00384-004-0615-3
18. Tursi A, Inchingolo CD, Picchio M, Elisei W, Mangiola F, Gasbarrini G. Histopathology of segmental colitis associated with diverticulosis resembles inflammatory bowel diseases. *J Clin Gastroenterol* 2015;49:350-351. doi:10.1097/MCG.0000000000000268
19. Galetin T, Galetin A, Vestweber KH, Rink AD. Systematic review and comparison of national and international guidelines on diverticular disease. *Int J Colorectal Dis* 2018;33:261-272. doi:10.1007/s00384-017-2960-z
20. Stollman N, Smalley W, Hirano I; AGA Institute Clinical Guidelines Committee. American Gastroenterological Association Institute Guideline on the Management of Acute Diverticulitis. *Gastroenterology* 2015;149:1944-1949. doi:10.1053/j.gastro.2015.10.003
21. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States part II: lower gastrointestinal diseases. *Gastroenterology* 2009;136:741-754. doi:10.1053/j.gastro.2009.01.015
22. Tursi A, Brandimarte G, Elisei W, et al. Randomised clinical trial: mesalazine and/or probiotics in maintaining remission of symptomatic uncomplicated diverticular disease--a double-blind, randomised, placebo-controlled study. *Aliment Pharmacol Ther* 2013;38:741-751. doi:10.1111/apt.12463
23. Tursi A, Elisei W, Giorgetti GM, et al. Detection of endoscopic and histological inflammation after an attack of colonic diverticulitis is associated with higher diverticulitis recurrence. *J Gastrointest Liver Dis* 2013;22:13-19.
24. Tursi A, Brandimarte G, Di Mario F, et al. Development and validation of an endoscopic classification of diverticular disease of the colon: the DICA classification. *Dig Dis* 2015;33:68-76. doi:10.1159/000366039
25. Tursi A, Brandimarte G, Di Mario F et al; DICA Collaborative Group. Predictive value of the Diverticular Inflammation and Complication Assessment (DICA) endoscopic classification on the outcome of diverticular disease of the colon: An international study. *United European Gastroenterol J* 2016;4:604-613. doi:10.1177/2050640615617636
26. Tursi A, Brandimarte G, Di Mario F, et al; DICA Italian Group. The "DICA" endoscopic classification for diverticular disease of the colon shows a significant interobserver agreement among community endoscopists. *J Gastrointest Liver Dis* 2019;28:23-27. doi:10.15403/jgld.2014.1121.281.dic
27. Yamada E, Kuriyama H, Uchida E, et al. Association between endoscopic findings related to colonic diverticula and bowel habits: A multicenter study in Japan. *J Gastroenterol Hepatol* 2017;32:1938-1942. doi:10.1111/jgh.13805
28. Lahat A, Necula D, Yavzori M, et al. Prolonged recurrent abdominal pain is associated with ongoing underlying mucosal inflammation in patients who had an episode of acute complicated diverticulitis. *J Clin Gastroenterol* 2019;53:e178-e185. doi:10.1097/MCG.0000000000000980
29. Järbrink-Sehgal ME, Rassam L, Jasim A, et al. Diverticulosis, Symptoms and Colonic Inflammation: A Population-Based Colonoscopy Study. *Am J Gastroenterol* 2019;114:500-510. doi:10.14309/ajg.000000000000113
30. Tursi A, Brandimarte G, Di Mario F, et al. P02.12 Predictive value of the "DICA" endoscopic classification on the outcome of diverticular disease of the colon: a 1-year analysis from the international, multicenter, prospective study. *Dig Liver Dis* 2019;51 (Suppl 2):e152-e153. doi:10.1016/S1590-8658(19)30297-X
31. Sartelli M, Moore FA, Ansaloni L, et al. A proposal for a CT driven classification of left colon acute diverticulitis. *World J Emerg Surg* 2015;10:3. doi:10.1186/1749-7922-10-3
32. Bates DDB, Fernandez MB, Ponchiardi C, et al. Surgical management in acute diverticulitis and its association with multi-detector CT, modified Hinchey classification, and clinical parameters. *Abdom Radiol (NY)* 2018;43:2060-2065. doi:10.1007/s00261-017-1422-y
33. Mora López L, Flores Clotet R, Serra Aracil X, Montes Ortega N, Navarro Soto S. The use of the modified Neff classification in the management of acute diverticulitis. *Rev Esp Enferm Dig* 2017;109:328-334. doi:10.17235/reed.2017.4738/2016
34. Ambrosetti P. Value of CT for acute left-colonic diverticulitis: the surgeon's view. *Dig Dis* 2012;30:51-55. doi:10.1159/000335717
35. Shin S, Kim D, Kang UR, Yang CS. Impact of CT imaging on predicting the surgical management of acute diverticulitis. *Ann Surg Treat Res* 2018;94:322-329. doi:10.4174/ast.2018.94.6.322
36. Ambrosetti P. Acute left-sided colonic diverticulitis: clinical expressions, therapeutic insights, and role of computed tomography. *Clin Exp Gastroenterol* 2016;9:249-257. doi:10.2147/CEG.S110428
37. Flor N, Maconi G, Sardanelli F, et al. Prognostic Value of the Diverticular Disease Severity Score Based on CT Colonography: Follow-up in Patients Recovering from Acute Diverticulitis. *Acad Radiol* 2015;22:1503-1509. doi:10.1016/j.acra.2015.08.022
38. Cuomo R, Barbara G, Pace F, et al. Italian consensus conference for colonic diverticulosis and diverticular disease. *United European Gastroenterol J* 2014; 2:413-442. doi:10.1177/2050640614547068
39. Lameris W, Van Randen A, Bipat S, et al. Graded compression ultrasonography and computed tomography in acute colonic diverticulitis: meta-analysis of test accuracy. *Eur Radiol* 2008;18:2498-2511. doi:10.1007/s00330-008-1018-6

- 
40. Dirks K, Calabrese E, Dietrich CF, et al. EFSUMB Position Paper: Recommendations for Gastrointestinal Ultrasound (GIUS) in Acute Appendicitis and Diverticulitis. *Ultraschall Med* 2019;40:163-175. doi:[10.1055/a-0824-6952](https://doi.org/10.1055/a-0824-6952)
41. Van Randen A, Lameris W, van Es HW, et al. A comparison of the accuracy of ultrasound and computed tomography in common diagnoses causing acute abdominal pain. *Eur Radiol* 2011;21:1535-1545. doi:[10.1007/s00330-011-2087-5](https://doi.org/10.1007/s00330-011-2087-5)