

CLINICAL STUDY

Fecal calprotectin as a promising marker of inflammatory diseases

Paduchova Z, Durackova Z

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty, Comenius University, Bratislava, Slovakia. zuzik78@gmail.com

Abstract: *Background:* Calprotectin is a calcium binding protein present predominantly in neutrophils with anti-microbial and antiproliferative activities. Calprotectin concentration is higher in feces than in plasma and significantly increased levels of fecal calprotectin (FC) were found in patients with bowel inflammation disease (IBD). *Methods:* Nineteen out-patients with IBD, comprising 14 Crohn's disease (CD) individuals and 5 ulcerative colitis (UC) patients, and 5 healthy volunteers were investigated. Fecal calprotectin was analyzed by ELISA. *Results:* We found that patients with IBD had significantly higher concentration of FC than in healthy children (FC median 1076.7 vs 19.5 µg/g of stool, $p=0.0053$). We determined higher level of FC in patients with CD than in UC (1132.4 vs 490.98 µg/g of stool), but not statistically significant. *Conclusion:* It has been proved that FC represents a surrogate marker of neutrophils influx into the bowel lumen; hence it can be regarded as a simple and non-invasive marker of intestinal inflammation (Tab. 2, Fig. 1, Ref. 38). Full Text (Free, PDF) www.bmj.sk.
Key words: calprotectin, inflammatory bowel disease (IBD), Crohn's disease.

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic condition marked by recurrent episodes of inflammation in the gastrointestinal tract. The anatomic location and degree of inflammation determine the predominant symptoms that include rectal bleeding, diarrhea and abdominal pain (1). Inflammatory activity is not directly observable by patients or physicians and many methods have been developed to quantify the severity and extent of this inflammation. To these methods clinical, serological and hematological, radiological, radio isotopic technique, endoscopic and histological investigations belong. Clinical examinations include indexes, such as Clinical Activity Index (CAI), Crohn's Disease Activity Index (CDAI) or Harvey Bradshaw Index, which have been rigorously developed and validated in clinical trials (2, 3, 4), but they are awkward to use in clinical practice and still rely heavily on subjective patient symptoms. Blood tests, such as erythrocyte sedimentation rate (ESR), orosomucoid, C-reactive protein (CRP), platelet and white cell counts, some cytokines such as IL-6, TNF- α and IL-1 β reflecting systemic consequences of inflammation, have been proposed as predictors and/or markers of clinical relapse of IBD with varying degrees of success.

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty, Comenius University, Bratislava, Slovakia

Address for correspondence: Z. Paduchova, RND, PhD, Dept of Medical Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty, Comenius University, Sasinkova 2, SK-811 08 Bratislava, Slovakia. Phone: +421.2.59357411

Acknowledgement: This study was supported by VEGA grant No.1/4310/07, grant AV 2007/16-UK-01 Ministry of Education of SR, grant GUK 28/2008 and by Mind and Health, civil association.

However, these are non-specific markers for inflammation of the gastrointestinal tract (5) except for CRP and ESR that have been used as markers to diagnose and to predict the activity of inflammatory disease. Imaging studies such as USG screening, CT, MRI scans, barium enemas, enteroclysis and irrigography can be useful in localizing intestinal inflammation, but these studies often are expensive, have suboptimal sensitivity and/or specificity, and may be invasive or expose the patient to ionizing radiation. Radioisotopically labeled compounds such as labeled white cells, red blood cells and proteins provided quantitative and functional data and were event specific (blood loss, inflammation, etc.) but non-specific for disease. The current gold standard for assessing intestinal inflammation is endoscopy with biopsies. This technique allows visual inspection of the gastrointestinal tract, and mucosal biopsy specimens can be obtained for histological examination. The location, extent, and severity of disease can be established with this procedure, but it is invasive, it cannot examine the entire gastrointestinal tract. This examination is painful and requires both a skilled operator and an uncomfortable preparatory regimen (6). It is evident that a simple, rapid, sensitive, specific, inexpensive, non-invasive marker to detect and monitor intestinal inflammation in IBD is needed. Fecal calprotectin (FC) could meet these requirements.

Structure of calprotectin

Calprotectin is a protein with molecular weight 36 kD and it was first isolated from granulocytes in 1980 and named L1 protein (7). The name calprotectin comes from the fact that it binds calcium and it has antimicrobial properties. In the literature it

Tab. 1. Fecal calprotectin concentration in healthy adults, children and infants as well as in adults and children with IBD.

Literature	Year	Age	Count of patients (n)			Calprotectin Median (µg/g)		
			H	CD	UC	H	CD	UC
Roseth et al	1997	adults	124		62	30		340
Ton et al	2000	adults	59			26		
Tibble et al	2000	adults	56	116		10	455	
Summerton et al	2002	adults	28	4	10	23	164	267
Thjodleifsson et al	2003	adults	163	49		20	235	
Carroccio et al	2003	adults	10	10		20	320	
Costa et al	2003	adults	34	49	82	11	231	167
Poullis et al	2004	adults	320			27		
Wassell et al	2004	adults	27	25		9,3	230	
Dolwani et al	2004	adults	56	25		10	227	
Costa et al	2005	adults		38	41		220	151
Bunn et al	2001	children	31	21	16	11	70	58
Olafsdottir et al	2002	children	24	17		40	293	
Fagerberg et al	2003	children	117			17		
Carroccio et al	2003	children	10	8		15	260	
Berni Canani et al	2004	children	76			28		
Nissen et al	2004	children	21	18	17	237		
Bremner et al	2005	children		43		337		
Berni Canani et al	2006	children		17	10		305	340
Olafsdottir et al	2002	infants (6 weeks)	27			278		
Rugtveit et al	2002	infants (6 weeks)	20			264		
Campeotto et al	2004	infants (1 week)	69			167		
Nissen et al	2004	infants (1–2 weeks)	16			235		

H – healthy volunteers, CD – Crohn's disease, UC – ulcerative colitis.

has several synonyms (complex of S100A8 and S100A9 proteins, L1L and L1H proteins, macrophage inhibitory factor-related protein MRP8/14, calgranulin A/B) (8). Calprotectin is a heterocomplex of two subunits (S100A8 and S100A9), it belongs to the S100 family of calcium-binding proteins (9). It comprises two heavy chains of 14 kD (L1H, MRP14) and one light chain of 8 kD (L1L, MRP8), which are non-covalently linked (10, 11).

Occurrence of calprotectin

Calprotectin concentration in neutrophils is abundant and constitutes of about half (30–60 % according to various authors) of total cytosolic proteins (12). Calprotectin is secreted extracellularly from stimulated neutrophils (13) and monocytes (14), or is released as a result of cell disruption or death (15). It is also found on the membrane of non-keratinizing squamous epithelia, and occasionally, in kidney tubules. Some mucosal epithelial cells express calprotectin in the cytoplasm constitutively (16). It is reported that skin and epithelial cells produce calprotectin only during inflammatory process. During microbial invasion calprotectin is produced and excreted out of epithelial cells. Calprotectin concentrations detected in colon of the healthy individuals are several times higher than in serum/plasma. It is related to two facts. Firstly, granulocytes migrating into the gut lumen are subjected to cytolysis with release of calprotectin and secondary, a continuous contact of intestinal mucosa with physiological bacterial flora activates epithelial cells to produce calprotectin (17). Calprotectin is resistant to colonic bacterial degradation. The

soluble form of calprotectin is possible to detect in plasma, serum, urine, saliva, intestinal fluid and feces (18).

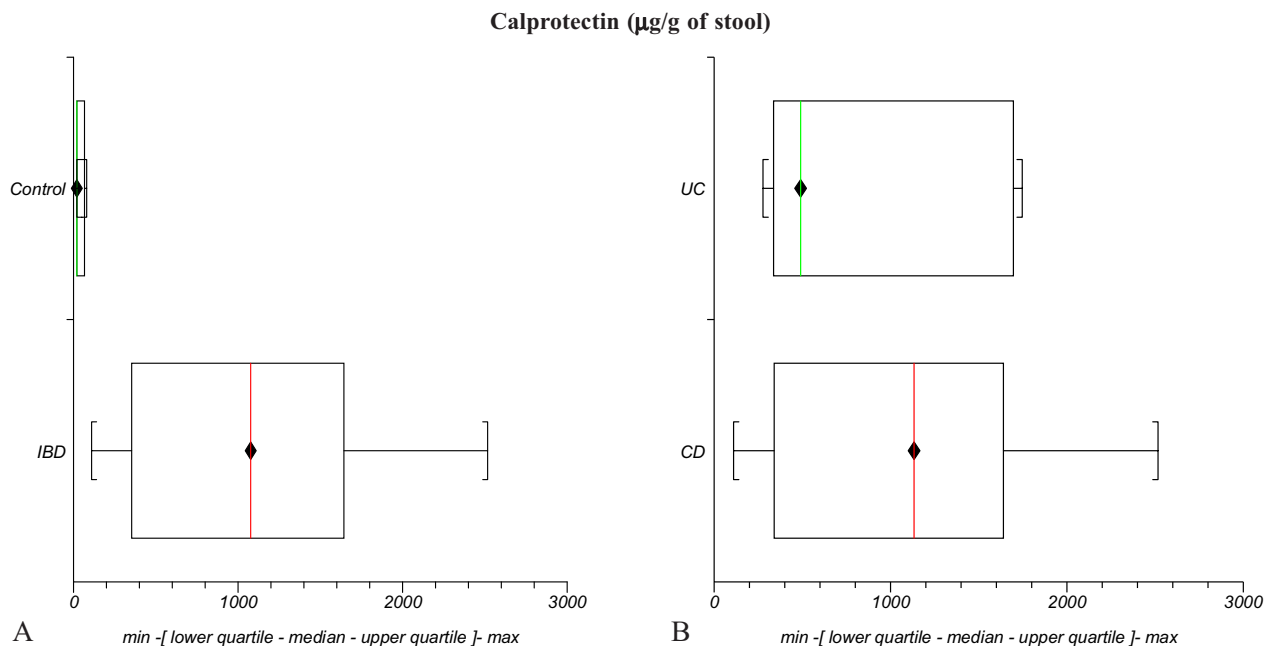
Biological functions of calprotectin

Calprotectin is a multipotent biologically active molecule (10). It appears to play a regulatory role in the inflammatory process (19) and functions in both an antiproliferative capacity (20) and antimicrobial capacity against bacteria, fungi and candida (21, 22, 23). It is reported that calprotectin antimicrobial activity is executed by at least three mechanisms. Firstly, it has a direct effect on microorganisms by means of zinc binding, what inhibits MMPs (matrix metalloproteinases), zinc-dependent enzymes that are important in angiogenesis, wound healing, inflammation, cancer, and tissue destruction (15, 24, 25). This activity is directed both against bacteria outside and inside cells after fagocytosis (24). Secondly,

Tab. 2. Reference values of fecal calprotectin in healthy children and children with IBD.

Calprotectin level	Clinical state
<50 µg Calprotectin/g stool	Normal
50–100 µg Calprotectin/g stool	Moderate GI inflammation
>100 µg Calprotectin/g stool	Significant GI inflammation
>250 µg Calprotectin/g stool	Mild to moderate IBD activity
>500 µg Calprotectin/g stool	Severe IBD activity

IBD – Inflammatory Bowel Disease, GI – gastrointestinal



Levels of fecal calprotectin in healthy controls (median 19.5 $\mu\text{g/g}$ of stool, range 19.5–79.4 $\mu\text{g/g}$ of stool, $n=5$) and children with IBD (median 1076.7 $\mu\text{g/g}$ of stool, range 109.9–2517.5 $\mu\text{g/g}$ of stool, $n=19$) (A), and in patients with CD (median 1132.4 $\mu\text{g/g}$ of stool, range 109.9–2517.5 $\mu\text{g/g}$ of stool, $n=14$) and UC (median 490.98 $\mu\text{g/g}$ of stool, range 275.3–1746.1 $\mu\text{g/g}$ of stool, $n=5$) (B).

Fig. 1. Comparison of fecal calprotectin concentrations in healthy controls and children with Inflammatory Bowel Disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC).

calprotectin presents similar properties as Neutrophil Inhibiting Factor (NIF); it promotes migration of neutrophils into an inflammatory area (17). Finally, calprotectin increases neutrophil's ability to fagocytosis. This triple action is especially effective in bactericidal and fungicidal activity of neutrophils (15, 24).

Calprotectin as a marker of inflammation

As we have mentioned above, calprotectin can be detected in plasma, body fluids, tissue, and feces and in other biological materials.

Plasma calprotectin levels are increased in many types of infectious or organic diseases and can differentiate between viral illnesses and bacterial infections. Its levels in plasma correlated with the disease activity and joint inflammation in rheumatoid arthritis. In healthy subjects plasma calprotectin levels are 0.1–0.6 mg/l, in subjects with viral infections 0.1–1.4 mg/l and in subjects with bacterial infections 0.6–11 mg/l (26).

Serum calprotectin can be a very sensitive marker of complications in organ transplantation and in combination with other inflammatory markers can be extremely specific for bacterial systemic infections (27).

Tissue calprotectin-producing granulocytes, monocytes and squamous epithelial cells can be detected by immunofluorescence or immunohistochemistry. This type of calprotectin can be used in patients with CD and UC. Histological assessment of intesti-

nal biopsies is painful, but in spite of that it is a vital part of the diagnosis of these conditions (28).

Calprotectin can be extracted and quantified from fecal samples. Elevated concentrations of calprotectin in feces have been measured in patients with colorectal cancer, IBD and bacterial infections in the gastrointestinal tract. It is a useful marker of bowel inflammation because it is markedly resistant to proteolytic degradation in the presence of calcium (29). Calprotectin reflects disease activity in IBD and can be used to monitor the response to treatment and detect relapses (30). In patients with IBD significantly higher concentrations of fecal calprotectin were determined in both adults and children in comparison to healthy controls (Tab. 1) (6). The reference values are similar for both groups (Tab. 2) (17). It is well known that calprotectin levels are elevated in healthy infants (≤ 10 weeks of age) (Tab. 1) in comparison to healthy children or adults (31). It has been shown that calprotectin concentrations increase with age in adults (32). Calprotectin levels significantly correlate with endoscopy, histological assessment, intestinal permeability, and clinical activity index CDAI and fecal excretion of ^{111}In -labeled neutrophils (33, 34).

Determination of fecal calprotectin

Determination of fecal calprotectin (FC) by an enzyme immunoassay (ELISA) specific for calprotectin is based upon prepa-

ration of an extract in a closed tube. The immunoassay use microtiter wells coated with polyclonal antibodies against calprotectin. The rabbit antibodies used in test react with at least six different epitopes on calprotectin which will ensure a positive signal even if some epitopes are damaged or hidden due to complex formation with other substances in the stool (35).

FC represents a surrogate marker of neutrophils influx into the bowel lumen; hence it can be regarded as simple, non-invasive and high sensitive marker of intestinal inflammation (36). Calprotectin measurement can help to identify UC and colonic CD patients at higher risk of clinical relapse (37, 38). Based on convincing studies and clinical experience over several years it is established as a routine test in Norway, Austria, Czech Republic and several centers in the UK.

The aim of this study is to summarize all information on calprotectin, to show its applications in several countries and to show our preliminary results in IBD children. We make an effort to establish calprotectin determination as a routine test in Slovakia.

Materials and methods

Patients: Nineteen outpatients – 11 boys and 8 girls – with IBD, comprising 14 Crohn's disease (CD) patients and 5 ulcerative colitis (UC) individuals, age 12–18 years (average age 15.6±0.5) were investigated. The control group was represented by healthy children (2 boys and 3 girls) of the similar age (average age 13.4±1.2).

The Ethical Committee of the Child University Hospital approved the study. Parents gave a written consent for participation of their children in the study.

Methods: Stool samples (1–5 g) were collected in suitable plastic containers and delivered to laboratory for processing. At room temperature FC is stable for at least 4 days (maximally 7 days). Since calprotectin is very stable in stool, patients can collect stool sample at home and send to the laboratory by ordinary mail. Subsequently stool is stored at -20 °C and calprotectin is stable and reproducible for 1 year. After thawing the determination of FC is based upon preparation of an extract of about 100 milligrams feces mixed with about 5 ml of extraction buffer in a closed tube. After centrifugation, a sample from the supernatant is tested by an enzyme immunoassay (ELISA) specific for calprotectin (Calpro test, Novatec Immundiagnostica GmbH, Germany) at 450 nm, according to the manufacturer protocol. Calprotectin was expressed in micrograms per gram of stool. According to the manufacturer, calprotectin level above 50 µg/g is pathological.

Statistical analysis

Median and interquartile range are given for data showing departures from normality (according to Shapiro-Wilks test). Statistical analysis (Wilcoxon matched pairs signed rank test) was performed using the statistical program StatsDirect 2.3.7 (StatsDirect Sales, Sale, Cheshire M33 3UY, UK). Significance was defined as $p < 0.05$. Graphical representation of data was made using program StatsDirect 2.3.7.

Results and discussion

We found out that patients with IBD had significantly higher concentration of FC than healthy children (median was 1076.7 vs 19.5 µg/g of stool, $p=0.0053$) (Fig. 1A). We determined also higher level of FC in patients with CD in comparison to UC (1132.4 vs 490.98 µg/g of stool, $p=0.411$), but not statistically significant (Fig. 1B).

Our results are in accord with levels of FC published by Konikoff and Denson (6). They showed significantly higher concentrations of FC in both adults and children in comparison to healthy controls (Tab. 1).

References

1. Angriman I, Scarpa M, D'Inca R et al. Enzymes in feces: Useful markers of chronic inflammatory bowel disease. *Clin Chim Acta* 2007; 381: 63–68.
2. Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. *Lancet* 1980; 1: 514.
3. Hyams JS, Ferry GD, Mandel FS et al. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr* 1991; 12 (4): 439–447.
4. Hyams J, Markowitz J, Otley A et al. Evaluation of the pediatric Crohn disease activity index: a prospective multicenter experience. *J Pediatr Gastroenterol Nutr* 2005; 41 (4): 416–421.
5. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut* 2006; 55: 426–431.
6. Konikoff MR, Denson LA. Role of calprotectin as a biomarker of intestinal inflammatory bowel disease. *Inflamm Bowel Dis* 2006; 12: 524–534.
7. Fagerhol MK, Dale I, Andersson I. Release and quantitation of a leucocyte derived protein (L1). *Scand J Haematol* 1980; 24: 393–398.
8. Dale I, Fagerhol MK, Naesgaard I. Purification and partial characterization of highly immunogenic human leukocyte protein, the L1 antigen. *Eur J Biochem* 1983; 134: 1–6.
9. Kligman D, Hilt DC. The S100 protein family. *Trends Biochem Sci* 1988; 13: 437–443.
10. Fagerhol MK, Andersson KB, Naess-Andresen CF et al. Calprotectin (The L1 leukocyte protein). 187–210. In: Smith VL and Dedman JR (Eds). *Stimulus response coupling: The role of intracellular calcium-binding proteins*. CRC Press; Boca Raton, 1990.
11. Naess-Andresen CF, Egelanddal B, Fagerhol MK. Calcium binding and concomitant changes in the structure and heat stability of calprotectin (L1 protein). *J Clin Pathol Mol Pathol* 1995; 316: M278–284.
12. Hessian PA, Edgeworth J, Hogg N. MRP-8 and MRP-14, two abundant Ca^{2+} -binding proteins of neutrophils and monocytes. *J Leukoc Biol* 1993; 53: 197–204.
13. Boussac M, Garin J. Calcium-dependent secretion in human neutrophils: a proteomic approach. *Electrophoresis* 2000; 21: 665–672.
14. Rammes A, Roth J, Goebeler M, Klempt M, Hartmann M, Sorg C. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J Biol Chem* 1997; 272 (14): 9496–9502.

15. **Voganatsi A, Panyutich A, Miyasaki KT, Murthy RK.** Mechanism of extracellular release of human neutrophil calprotectin complex. *J Leukoc Biol* 2001; 70: 130–134.
16. **Brandtzaeg P, Dale I, Fagerhol MK.** Distribution of a formalin-resistant myelomonocytic antigen (Li) in human tissues. I. Comparison with other leukocyte markers by paired immunofluorescence and immunoenzyme staining. *AmER J Clin Pathol* 1987; 87: 681–699.
17. **Tibble JA, Bjarnason I.** Non-invasive investigation of inflammatory bowel disease. *World J Gastroenterol* 2001; 7 (4): 460–465.
18. **Tibble JA, Bjarnason I.** Fecal calprotectin as an index in intestinal inflammation. *Drugs Today (Brac)* 2001; 37 (2): 85–96.
19. **Brun JG, Ulvestad E, Fagerhol MK, Jonsson R.** Effect of human calprotectin (L1) on in vitro immunoglobulin synthesis. *Scand J Immunol* 1994; 40: 675–680.
20. **Yui S, Mikami M, Tsurumaki K, Yamazaki M.** Growth-inhibitory and apoptosis inducing activities of calprotectin derived from inflammatory exudate cells on normal fibroblasts: regulation by metal ions. *J Leukoc Biol* 1997; 61: 50–57.
21. **Steinbakk M, Naess-Andersen C-F, Lingaas E et al.** Antimicrobial actions of calcium binding leucocyte Li protein, calprotectin. *Lancet* 1990; 336: 763–765.
22. **Sohnle PG, Collins-Lech C, Wiessner JH.** The zinc-reversible antimicrobial activity of neutrophil lysates and abscess fluid supernatants. *J Infect Dis* 1991; 164: 136–142.
23. **Hahn BL, Sohnle PG.** Resistance of zinc-supplemented *Candida albicans* cells to the growth inhibitory effect of calprotectin. *J Infect Dis* 1995; 171: 1289–1294.
24. **Levy O.** Antimicrobial proteins and peptides of blood: templates for novel antimicrobial agents. *Blood* 2000; 96: 2664–2672.
25. **Fagerhol MK.** Calprotectin, a faecal marker of organic gastrointestinal abnormality. *The Lancet* 2000; 356: 1783–1784.
26. **Poullis A, Foster R, Mendall MA, Fagerhol MK.** Emerging role of calprotectin in gastroenterology. *J Gastroenterol Hepatol* 2003; 18: 756–762.
27. **Burkhardt K, Radespiel-Troger M, Rupprecht HD et al.** An increase in myeloid-related protein serum levels precedes acute renal allograft rejection. *J Amer Soc Nephrol* 2001; 12: 1947–1957.
28. **Bjerke K, Halstensen TS, Jahnsen F, Pulford K, Brandtzaeg P.** Distribution of macrophages and granulocytes expressing L1 protein (calprotectin) in human Peyer's patches compared with normal ileal lamina propria and mesenteric lymph nodes. *Gut* 1993; 34: 1357–1363.
29. **Fagerhol MK.** Nomenclature for proteins: is calprotectin a proper name for the elusive myelomonocytic protein? *J Gastroenterol* 1996; 49: 74–79.
30. **Aadland E, Fagerhol MK.** Faecal calprotectin: a marker of inflammation throughout the intestinal tract. *Eur J Gastroenterol Hepatol* 2002; 14: 823–825.
31. **Olafsdottir E, Aksnes L, Fluge G, Berstad A.** Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta Paediatr* 2002; 91: 45–50.
32. **Poullis A, Foster R, Shetty A, Fagerhol MK, Mendall MA.** Bowel inflammation as measured by fecal calprotectin: a link between lifestyle factors and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2004; 13 (2): 279–284.
33. **Roseth AG, Aadland E, Jahnsen J, Raknerud N.** Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997; 58: 176–180.
34. **Tibble JA, Sigthorsson G, Bridger S et al.** Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000; 119: 15–22.
35. **Ton H, Brandsnes O, Dale S, Holtlund J, Skuibina E, Schjonsby H, Johne B.** Improved assay for fecal calprotectin. *Clin Chim Acta* 2000; 292: 41–54.
36. **Wagner M, Peterson CGB, Ridefelt P, Sangfelt P, Carlson M.** Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J Gastroenterol* 2008; 14 (36): 5584–5589.
37. **D, Inca R, Del Pont E, DiLeo V et al.** Can calprotectin predict relaps risk in inflammatory bowel disease? *Amer J Gastroenterol* 2008; 103 (8): 2007–2014.
38. **Diamanti A, Colistro F, Basso MS et al.** Clinical role of calprotectin assay in determining histological relapses in children affected by inflammatory bowel diseases. *Inflamm Bowel Dis* 2008; 14 (9): 1229–1235.

Received May 22, 2009.

Accepted June 26, 2009.