



Peter L Lakatos, MD, PhD, Assistant Professor, Series Editor

Serologic and laboratory markers in prediction of the disease course in inflammatory bowel disease

Marla Cindy Dubinsky

Marla Cindy Dubinsky, Pediatric IBD Center, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

Author contributions: Dubinsky MC solely contributed to this paper.

Correspondence to: Dr. Marla Cindy Dubinsky, Pediatric IBD Center, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States. marla.dubinsky@cshs.org
Telephone: +1-310-4237100 Fax: +1-310-4231402

Received: November 28, 2009 Revised: January 7, 2010

Accepted: January 14, 2010

Published online: June 7, 2010

Abstract

The search for biologic markers that can assess the natural history and perhaps predict the course of individual's disease including response to treatments over time has become an important focus of inflammatory bowel disease research. The knowledge of an individual's prognosis can help physicians and patients make important management decisions and aid communication on risk and benefits of disease and treatment.

© 2010 Baishideng. All rights reserved.

Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Perinuclear anti-neutrophil antibody; Anti-*Saccharomyces cerevisiae* antibody; Pouchitis; Internal penetrating; Fibrostenosing

Peer reviewer: Kazuichi Okazaki, Professor, Third Department of Internal Medicine, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi City, Osaka, 570-8506, Japan

Dubinsky MC. Serologic and laboratory markers in prediction of the disease course in inflammatory bowel disease. *World J Gastroenterol* 2010; 16(21): 2604-2608 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v16/i21/2604.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i21.2604>

INTRODUCTION

The ideal serological and laboratory marker should be able to identify individuals at risk for the disease and should be disease specific. Moreover it should be able to detect disease activity and monitor the effect of treatment; and finally it should have a predictive value towards relapse or recurrence of the disease. Predicting disease course however has now been expanded beyond just disease recurrence, but perhaps more importantly to include predictors of disease complications including surgery. This topic highlight will focus on the prognostic role of serological and laboratory markers and the importance of phenotype stratification in inflammatory bowel disease (IBD) patients.

SEROLOGICAL IMMUNE MARKERS

The search for the underlying trigger of the abnormal intestinal inflammatory reaction characteristic of IBD has led to the discovery of antibodies present specifically in the blood of patients with Crohn's disease and/or ulcerative colitis (UC)^[1-6]. Perinuclear anti-neutrophil antibody (pANCA) is noted for its association with UC or a UC like phenotype^[7-13]. Anti-*Saccharomyces cerevisiae* antibody (ASCA) is another important antibody marker that is present in the blood of individuals with IBD^[7-9]. ASCA is part of the family of anti-glycan (carbohydrate) antibodies. Other anti-glycan antibodies, antibodies against laminaribioside (ALCA) and chitobioside (ACCA) have been studied in IBD^[14]. In addition to the anti-glycan antibodies, 3 additional markers representative of microbial driven immune responses have been identified; antibodies to the *Escherichia coli* (*E. coli*) outer-membrane porin C (OmpC), the *Pseudomonas fluorescens* CD related protein [anti-CD related bacterial sequence (I2)] and the CBir1 flagellin^[15,16]. As seen with the genetic and clinical heterogeneity of CD, studies have shown immune response (immune phenotype) heterogeneity exists among

CD patients. Landers *et al*^[15] analyzed immune response heterogeneity in 330 patients and demonstrated that CD patients could be clustered into 4 distinct groups depending on their immune response patterns to microbial or auto-antigens. One cluster was ASCA, a second was antibodies to OmpC and I2, the third pANCA and the fourth was low or no immune response to any tested antigens. Immune reactivity to CBir1 may further define CD phenotypes in that anti-CBir1 expression is present in 40%-44% of pANCA positive CD patients *vs* only 4% in pANCA positive UC patients. This difference may denote a unique etiopathogenic mechanism of disease that helps to further stratify patients based on immunogenetic phenotypes.

Vasiliauskas *et al*^[17] introduced the notion of immune response stratification when he first reported that high ASCA levels were found to be associated with fibrostenosing (FS) and internal-penetrating (IP) disease as well as the need for small bowel surgery. Another cross sectional study demonstrated that patients who were ASCA IgA or IgG positive were 8.5 times and 5.5 times more likely to undergo early surgery (within 3 years of diagnosis) than ASCA IgA or IgG negative patients^[18]. Mow *et al*^[19] examined the association of multiple immune responses and disease phenotype. Reactivity to OmpC was independently associated with IP disease, while reactivity to anti-I2 was independently associated with FS disease and the need for surgery. Both the presence and magnitude of the immune response was associated with more aggressive disease behaviors. A similar study in a Scottish CD cohort reported that the cumulative reactivity to ASCA, I2 and OmpC was associated with small bowel complications^[20]. Antibodies to CBir1 were examined in a later study and were found to be independently associated with small bowel disease, IP and FS disease^[16]. Xue *et al*^[21] demonstrated that reactivity to ASCA, OmpC and CBir1 was associated with early disease onset, FS and IP disease and the need for surgery. The presence and magnitude of the serological response to anti-glycan antibodies were found to be associated with complicated disease, disease duration, and need for surgery^[22]. Subsequently, increasing amount and level of antibody responses toward gASCA, ALCA, ACCA, AMCA, and OmpC were found to be associated with more complicated disease behavior ($P < 0.0001$) and need for surgery in CD ($P = 0.023$) in an eastern European IBD cohort^[23].

More recent pediatric cohort studies suggest that these markers are present in patients before a complication occurs and thus predictive of disease progression from uncomplicated to complicated disease state. Desir *et al*^[24] demonstrated that baseline ASCA reactivity was associated with a more relapsing course in a pediatric CD cohort (OR = 2.9; 95% CI: 1.33-6.35). A multi-center study examined the association of ASCA, anti-I2, anti-OmpC, and anti-CBir1 reactivity with disease course in close to 800 pediatric CD patients^[25]. The frequency of development of disease complications (IP and or FS) increased in parallel with reactivity to increasing numbers

of antigens. The odds ratio for the development of IP disease was 5.0 and 9.5 for children with reactivity to 2 and 3 antigens, respectively. Furthermore, survival analysis demonstrated that reactivity to at least one microbial antigen was associated with the development of surgery faster as compared to patients negative for all markers suggesting that these markers may predict more aggressive disease behaviors. Amre *et al*^[26] also studied a cohort of pediatric CD patients who had sera drawn at diagnosis and were studied for the subsequent development of complications of their disease. Survival analysis revealed that the time to first complication was more rapid for ASCA positive patients than those who were ASCA negative. Moreover the relative risk (RR) of a recurrent complication (RR = 3.68) and needs for an additional surgery (RR = 1.95) was significantly higher in ASCA positive patients. As compared to the positive association between ASCA, antibodies to OmpC, I2 and CBir1 and disease complication, pANCA has been shown to be associated with a more benign, UC-like disease course and negatively associated with small bowel complicating disease^[17,27]. High pre-colectomy levels of pANCA (> 100 EU/mL) have been prospectively shown to be associated with the development chronic pouchitis all IBD patients undergoing IPAA^[28]. More recently, the same group reported that anti-CBir1 may accelerate the development of chronic pouchitis in the face of high pANCA levels^[29]. Melmed demonstrated that ASCA positivity and a family history of Crohn's disease was most predictive of CD of the pouch after IPAA^[30]. This information may not change the need for colectomy but the surgical procedure chosen and the post operative management would certainly be impacted by this prognostic information.

FECAL CALPROTECTIN AND C-REACTIVE PROTEIN

Other biomarkers such as fecal calprotectin and C-reactive protein (CRP) have been examined as non invasive tools to predict disease course but with a focus on relapse or disease recurrence. Calprotectin is an abundant neutrophil protein found in both plasma and stool that is markedly elevated in infectious and inflammatory conditions including IBD. Calprotectin constitutes approximately 5% of the total protein and 60% of the cytosolic protein in human neutrophils^[31]. Plasma calprotectin has been shown to increase 5 to 40 fold in infectious and inflammatory conditions^[32] and fecal calprotectin concentration is approximately six times that of normal plasma^[33]. The clinical course of most patients with IBD is typically marked by periods of remission with intermittent relapses. Because the timing of these relapses can be unpredictable, clinical symptoms more so than invasive tests are used to monitor disease activity. Most IBD relapses, however, come to medical attention after the inflammatory response has become well-established and clinical symptoms are worsening. Given that fecal calprotectin has been shown to correlate well with endoscopic and

histologic intestinal inflammation, its use as a predictor of relapse in patients with quiescent IBD has been examined. Tibble *et al*^[34] studied 43 patients with CD and 37 patients with UC in clinical remission for 1-4 mo as determined by clinical disease activity indices and Harvey Bradshaw index. These patients had fecal calprotectin measurements at baseline and were then followed for 1 year. Approximately half of the patients in each group relapsed during the 12 mo follow up period. The sensitivity and specificity of calprotectin for predicting relapse within the year in all patients with IBD were 90% and 83%, respectively by using a cut off of 50 mg/L. This cut off was associated with a 13-fold increased risk of relapse. These values were similar when patients with CD or UC were examined separately. Costa *et al*^[35] found nearly identical sensitivity and specificity of calprotectin for predicting relapse in UC, but their specificity of calprotectin in CD was 43%, markedly lower than the 83% reported by Tibble *et al*^[34] noted above. This large discrepancy may be explained by the fact that their study used a different assay and a lower cut-off of fecal calprotectin (150 µg/g, corresponding to 30 mg/L). A more recent study prospectively evaluated 97 patients with UC and 65 with CD in clinical remission for at least 6 mo^[36]. The cutoff level was set at 130 mg/kg of feces. Patients were followed up for 1 year after the test or until relapse. Fecal calprotectin was positive in 44 UC patients and 26 of them relapsed within a year, while 11 of 53 UC patients with a negative calprotectin relapsed within the year. Thirty CD patients had a positive calprotectin and 13 of them relapsed within a year, as did 7 of the 35 with a negative test result. A significant correlation emerged between a positive calprotectin and the probability of relapse in UC patients ($P = 0.000$). In CD patients, only cases of colonic CD showed a significant correlation between a positive calprotectin test and the probability of relapse, such that 6 colonic CD patients were positive for the calprotectin test and 4 relapsed ($P = 0.02$). A subsequent multicenter study demonstrated that fecal calprotectin concentrations in patients who suffered a relapse were higher than in nonrelapsing patients $P < 0.001$ ^[37]. Relapse risk was higher in patients having high (> 150 µg/g) calprotectin concentrations (30% *vs* 7.8%, $P < 0.001$). The area under the receiver operating characteristic curve to predict relapse using calprotectin determination was 0.73 (0.69 for UC and 0.77 for CD). Better results were obtained when only colonic CD disease or only relapses during the first 3 mo were considered (100% sensitivity).

CRP is one of the most important acute phase proteins in humans. Under normal circumstances CRP is produced by hepatocytes in low quantities but following an inflammatory stimulus, hepatocytes rapidly increase production of CRP under the influence of interleukin (IL)-6, tumour necrosis factor α , and IL-1 β . CRP has a short half life (19 h) compared with other acute phase proteins and will therefore rise early after the onset of inflammation and rapidly decrease after the stimulus is resolved. Although CRP is upregulated in most inflam-

matory diseases, including IBD, CD is associated with a strong CRP response and UC only a modest and sometimes absent CRP response^[38,39]. Moreover not all IBD patients mount a CRP response, even in CD and this must be kept in mind when measuring inflammatory markers in individual patients. It is unclear if this is due to differences in cytokine levels such as IL-6 or due to mucosal *vs* transmural disease differences among UC and CD or is genetically driven^[40-43]. CRP has been shown to be a good marker for predicting disease course and outcome in a number of diseases with cardiovascular disease and poor outcome after myocardial infarction as well as in multiple myeloma^[44-47]. Boirivant *et al*^[48] prospectively followed 101 CD outpatients with 50% having raised CRP which correlated well with disease activity. However, 1/3 of patients presented with active disease despite normal CRP and 1/3 had raised CRP but reported inactive disease. The likelihood of relapse after 2 years was higher in CD patients with an increased CRP compared with patients with normal CRP. Perhaps the best study to date on the predictability of CRP and other biomarkers was reported by the GETAID group who prospectively followed 71 CD patients with medically induced remission and measured laboratory markers (full blood count, CRP, ESR, α 1 antitrypsin, orosomucoid) every 6 wk^[49]. In total, 38 patients relapsed after a median follow up of 31 wk. In the end only two laboratory markers were predictive of relapse: CRP (20 mg/L) and ESR (15 mm). Patients with both markers positive had an 8 fold increased risk for relapse with a negative predictive value of 97%, suggesting that normal CRP and ESR has a very high probability of ruling out relapse in the preceding 6 wk. There are fewer studies examining the role of these markers in predicting UC recurrence or outcome. One study evaluated 49 severe hospitalized UC patients treated with steroids and/or cyclosporine. On day 3, a stool frequency of $> 8/d$ or 3-8 stools/d plus an increased CRP (0.45 mg/L) predicted with 85% certainty the need for colectomy^[50].

CONCLUSION

Research has fostered a novel approach to understanding the intricate relationship between genetic and clinical expression of disease. Serum immune markers hold the most promise in helping researchers better comprehend disease heterogeneity and natural history. Although our current gold standard diagnostic tests do not possess this capability, prospective research studies now suggest that IBD-specific genetic and antibody markers may serve as predictors of an individual's disease course. Although promising at predicting disease recurrence, larger studies are needed with more frequent biomarker testing as this may allow for early detection of active, perhaps sub clinical inflammation which may alter treatment decisions. Their role as non-invasive predictors of post-operative recurrence is currently under investigation and will be of interest to help guide post-operative maintenance strategies.

REFERENCES

- 1 **Duchmann R**, May E, Heike M, Knolle P, Neurath M, Meyer zum Büschenfelde KH. T cell specificity and cross reactivity towards enterobacteria, bacteroides, bifidobacterium, and antigens from resident intestinal flora in humans. *Gut* 1999; **44**: 812-818
- 2 **Vidrich A**, Lee J, James E, Cobb L, Targan S. Segregation of pANCA antigenic recognition by DNase treatment of neutrophils: ulcerative colitis, type 1 autoimmune hepatitis, and primary sclerosing cholangitis. *J Clin Immunol* 1995; **15**: 293-299
- 3 **Eggna M**, Cohavy O, Parseghian MH, Hamkalo BA, Clemens D, Targan SR, Gordon LK, Braun J. Identification of histone H1 as a cognate antigen of the ulcerative colitis-associated marker antibody pANCA. *J Autoimmun* 2000; **14**: 83-97
- 4 **Cohavy O**, Harth G, Horwitz M, Eggna M, Landers C, Sutton C, Targan SR, Braun J. Identification of a novel mycobacterial histone H1 homologue (HupB) as an antigenic target of pANCA monoclonal antibody and serum immunoglobulin A from patients with Crohn's disease. *Infect Immun* 1999; **67**: 6510-6517
- 5 **Seibold F**, Brandwein S, Simpson S, Terhorst C, Elson CO. pANCA represents a cross-reactivity to enteric bacterial antigens. *J Clin Immunol* 1998; **18**: 153-160
- 6 **Cohavy O**, Bruckner D, Gordon LK, Misra R, Wei B, Eggna ME, Targan SR, Braun J. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. *Infect Immun* 2000; **68**: 1542-1548
- 7 **Ruemmele FM**, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. *Gastroenterology* 1998; **115**: 822-829
- 8 **Quinton JF**, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastien B, Charrier G, Targan SR, Colombel JF, Poullain D. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998; **42**: 788-791
- 9 **Hoffenberg EJ**, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. *J Pediatr* 1999; **134**: 447-452
- 10 **Duerr RH**, Targan SR, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis. Comparison with other colitides/diarrheal illnesses. *Gastroenterology* 1991; **100**: 1590-1596
- 11 **Proujansky R**, Fawcett PT, Gibney KM, Treem WR, Hyams JS. Examination of anti-neutrophil cytoplasmic antibodies in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1993; **17**: 193-197
- 12 **Winter HS**, Landers CJ, Winkelstein A, Vidrich A, Targan SR. Anti-neutrophil cytoplasmic antibodies in children with ulcerative colitis. *J Pediatr* 1994; **125**: 707-711
- 13 **Oberstadt K**, Schaedel W, Weber M, Classen M, Deusch K. P-ANCA as a differential diagnostic marker in inflammatory bowel disease. *Adv Exp Med Biol* 1995; **371B**: 1313-1316
- 14 **Dotan I**, Fishman S, Dgani Y, Schwartz M, Karban A, Lerner A, Weishauss O, Spector L, Shtevi A, Altstock RT, Dotan N, Halpern Z. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006; **131**: 366-378
- 15 **Landers CJ**, Cohavy O, Misra R, Yang H, Lin YC, Braun J, Targan SR. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology* 2002; **123**: 689-699
- 16 **Targan SR**, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, Vasiliauskas E, Elson CO, Hershsberg RM. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005; **128**: 2020-2028
- 17 **Vasiliauskas EA**, Kam LY, Karp LC, Gaiennie J, Yang H, Targan SR. Marker antibody expression stratifies Crohn's disease into immunologically homogeneous subgroups with distinct clinical characteristics. *Gut* 2000; **47**: 487-496
- 18 **Forcione DG**, Rosen MJ, Kiesel JB, Sands BE. Anti-Saccharomyces cerevisiae antibody (ASCA) positivity is associated with increased risk for early surgery in Crohn's disease. *Gut* 2004; **53**: 1117-1122
- 19 **Mow WS**, Vasiliauskas EA, Lin YC, Fleshner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004; **126**: 414-424
- 20 **Arnott ID**, Landers CJ, Nimmo EJ, Drummond HE, Smith BK, Targan SR, Satsangi J. Sero-reactivity to microbial components in Crohn's disease is associated with disease severity and progression, but not NOD2/CARD15 genotype. *Am J Gastroenterol* 2004; **99**: 2376-2384
- 21 **Xue S**, Stempak JM, Elkadri AA, Greenberg GR, Walters TD, Griffiths AM, Steinhart H, Silverberg MS. Serological markers are associated with severity of disease and need for surgery in IBD patients. *Gastroenterology* 2006; **130**: S1303
- 22 **Ferrante M**, Henckaerts L, Joossens M, Pierik M, Joossens S, Dotan N, Norman GL, Altstock RT, Van Steen K, Rutgeerts P, Van Assche G, Vermeire S. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007; **56**: 1394-1403
- 23 **Papp M**, Altorjay I, Dotan N, Palatka K, Foldi I, Tumpek J, Sipka S, Udvardy M, Dinya T, Lakatos L, Kovacs A, Molnar T, Tulassay Z, Miheller P, Norman GL, Szamosi T, Papp J, Lakatos PL. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008; **103**: 665-681
- 24 **Desir B**, Amre DK, Lu SE, Ohman-Strickland P, Dubinsky M, Fisher R, Seidman EG. Utility of serum antibodies in determining clinical course in pediatric Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 139-146
- 25 **Dubinsky MC**, Kugathasan S, Mei L, Picornell Y, Nebel J, Wrobel I, Quiros A, Silber G, Wahbeh G, Katzir L, Vasiliauskas E, Bahar R, Otley A, Mack D, Evans J, Rosh J, Hemker MO, Leleiko N, Crandall W, Langton C, Landers C, Taylor KD, Targan SR, Rotter JI, Markowitz J, Hyams J. Increased immune reactivity predicts aggressive complicating Crohn's disease in children. *Clin Gastroenterol Hepatol* 2008; **6**: 1105-1111
- 26 **Amre DK**, Lu SE, Costea F, Seidman EG. Utility of serological markers in predicting the early occurrence of complications and surgery in pediatric Crohn's disease patients. *Am J Gastroenterol* 2006; **101**: 645-652
- 27 **Vasiliauskas EA**, Plevy SE, Landers CJ, Binder SW, Ferguson DM, Yang H, Rotter JI, Vidrich A, Targan SR. Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996; **110**: 1810-1819
- 28 **Fleshner PR**, Vasiliauskas EA, Kam LY, Fleshner NE, Gaiennie J, Abreu-Martin MT, Targan SR. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. *Gut* 2001; **49**: 671-677
- 29 **Fleshner P**, Ippoliti A, Dubinsky M, Vasiliauskas E, Mei L, Papadakis KA, Rotter JI, Landers C, Targan S. Both preoperative perinuclear antineutrophil cytoplasmic antibody and anti-CBir1 expression in ulcerative colitis patients influence pouchitis development after ileal pouch-anal anastomosis. *Clin Gastroenterol Hepatol* 2008; **6**: 561-568
- 30 **Melmed GY**, Fleshner PR, Bardakcioglu O, Ippoliti A, Vasiliauskas EA, Papadakis KA, Dubinsky M, Landers C, Rotter JI, Targan SR. Family history and serology predict Crohn's

- disease after ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum* 2008; **51**: 100-108
- 31 **Røseth AG**, Fagerhol MK, Aadland E, Schjønby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scand J Gastroenterol* 1992; **27**: 793-798
 - 32 **Bunn SK**, Bisset WM, Main MJ, Golden BE. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2001; **32**: 171-177
 - 33 **Fagerberg UL**, Lööf L, Merzoug RD, Hansson LO, Finkel Y. Fecal calprotectin levels in healthy children studied with an improved assay. *J Pediatr Gastroenterol Nutr* 2003; **37**: 468-472
 - 34 **Tibble JA**, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000; **119**: 15-22
 - 35 **Costa F**, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; **54**: 364-368
 - 36 **D'Incà R**, Dal Pont E, Di Leo V, Benazzato L, Martinato M, Lamboglia F, Oliva L, Sturniolo GC. Can calprotectin predict relapse risk in inflammatory bowel disease? *Am J Gastroenterol* 2008; **103**: 2007-2014
 - 37 **Gisbert JP**, Bermejo F, Pérez-Calle JL, Taxonera C, Vera I, McNicholl AG, Algaba A, López P, López-Palacios N, Calvo M, González-Lama Y, Carneros JA, Velasco M, Maté J. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009; **15**: 1190-1198
 - 38 **Pepys MB**, Druguet M, Klass HJ, Dash AC, Mirjah DD, Petrie A. Immunological studies in inflammatory bowel disease. *Ciba Found Symp* 1977; 283-304
 - 39 **Saverymuttu SH**, Hodgson HJ, Chadwick VS, Pepys MB. Differing acute phase responses in Crohn's disease and ulcerative colitis. *Gut* 1986; **27**: 809-813
 - 40 **Gross V**, Andus T, Caesar I, Roth M, Schölmerich J. Evidence for continuous stimulation of interleukin-6 production in Crohn's disease. *Gastroenterology* 1992; **102**: 514-519
 - 41 **Szalai AJ**, McCrory MA, Cooper GS, Wu J, Kimberly RP. Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. *Genes Immun* 2002; **3**: 14-19
 - 42 **Russell AI**, Cunninghamham DS, Shepherd C, Robertson CA, Whittaker J, Meeks J, Powell RJ, Isenberg DA, Walport MJ, Vyse TJ. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. *Hum Mol Genet* 2004; **13**: 137-147
 - 43 **Carlson CS**, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, Foranage M, Boerwinkle E, Gross M, Jaquish C, Nickerson DA, Myers RM, Siscovick DS, Reiner AP. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet* 2005; **77**: 64-77
 - 44 **Ridker PM**, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000; **342**: 836-843
 - 45 **Pearson TA**, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107**: 499-511
 - 46 **Danesh J**, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004; **350**: 1387-1397
 - 47 **Bataille R**, Boccadoro M, Klein B, Durie B, Pileri A. C-reactive protein and beta-2 microglobulin produce a simple and powerful myeloma staging system. *Blood* 1992; **80**: 733-737
 - 48 **Boirivant M**, Leoni M, Taricciotti D, Fais S, Squarcia O, Pallone F. The clinical significance of serum C reactive protein levels in Crohn's disease. Results of a prospective longitudinal study. *J Clin Gastroenterol* 1988; **10**: 401-405
 - 49 **Consigny Y**, Modigliani R, Colombel JF, Dupas JL, Mary JY. Biological markers of short term relapse in Crohn's disease (CD). *Gastroenterology* 2001; **20** suppl: A53
 - 50 **Travis SP**, Farrant JM, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MG, Jewell DP. Predicting outcome in severe ulcerative colitis. *Gut* 1996; **38**: 905-910

S- Editor Tian L L- Editor O'Neill M E- Editor Zheng XM