Laboratory Evaluation of Inflammatory Bowel Disease

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Introduction

Although clinical history and physical exam may raise suspicion of Crohn disease (CD) or ulcerative colitis (UC), a focused laboratory evaluation can facilitate further differentiation between inflammatory bowel disease (IBD) and noninflammatory bowel disease – in particular, distinguishing between IBD, infectious processes, and functional bowel disorders (Table 18.1). These blood and stool studies, in combination with clinical presentation (thorough history, including family history of IBD or other autoimmune conditions, and physical examination), can help determine which child may require more extensive or invasive testing, such as radiological and endoscopic evaluation to definitively diagnose IBD and provide information to facilitate IBD phenotype. Moreover, the blood and stool evaluations may also provide insight into the severity of disease, if indeed IBD (i.e., prognostication). The first part of this review will focus on the evaluation of blood tests in the work-up of a child with suspected IBD. Initially, the nonspecific markers of disease (e.g., anemia) and inflammation (e.g., C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) will be discussed. Subsequently, the more "specific" serological markers of IBD will be reviewed, and then, stool tests, which can be used to potentially delineate between IBD and non-IBD will be discussed.

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Blood Tests

Most clinicians, adult and pediatric, will agree that blood tests should be part of the initial screening process in children with symptoms compatible with UC or CD [1–6]. The specific blood evaluations performed should, at minimum, consist of a complete blood count, including white blood cell number with a differential, hemoglobin and hematocrit, and iron/red blood cell characteristics or indices such as mean corpuscular volume, as well as studies to further characterize iron deficiency including ferritin, total iron binding content (TIBC) and iron. In addition, liver biochemistries: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), albumin and total protein, and systemic inflammatory markers, such as ESR and CRP should be included in the initial laboratory evaluation of a child with suspected IBD [6, 7]. Although normal tests do not rule out the possibility of intestinal inflammation, if abnormalities are present, further diagnostic studies are generally warranted. In addition, serum biomarkers such as CRP and ESR can distinguish between quiescent and active disease and in some studies, elevations in these biomarkers have correlated with endoscopic evidence of mucosal disease [7]. As several of these parameters are included in the Pediatric Crohn Disease Activity Index (CDAI) (e.g., albumin, ESR), these blood tests may offer additional insight into disease activity, and potentially, severity [6, 8, 9].

Anemia

Anemia is a well-known complication of inflammatory bowel disease occurring in both UC[10] and CD [11–17]. Anemia is generally defined as a hemoglobin value <120 g/L. With respect to IBD, severe anemia is defined as a hemoglobin level <100 g/L. For reasons that are not well characterized, many patients with IBD are intolerant of oral iron replacement therapy or their anemia is refractory to such

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Table 18.1 Laboratory tests for suspected inflammatory bowel disease

Test	Findings	Significance
Complete blood count and differential	Anemia (microcytic, macrocytic, normocytic), thrombocytosis, leukocytosis	*Anemia:* Assess severity of blood loss, evaluate for iron and other macronutrient deficiencies. Reported prevalence 16–77% in Crohn disease and 9–67% in ulcerative colitis [16, 17] *Thrombocytosis:* Acute phase reactant, nonspecific measure of inflammation. Reported prevalence variable, occurring in up to 85% of patients with Crohn disease and 70% patients with ulcerative colitis [37, 38]
ESR and CRP	Elevation	Nonspecific markers of inflammation, potential role in assessing disease activity, predicting disease relapse and monitoring therapeutic response [47, 61]
Liver function tests	Hypoalbuminemia Elevated transaminases Elevated alkaline phosphatase/GGT	*Hypoalbuminemia:* Surrogate marker of nutrition, possibly indicative of decreased liver production (negative acute phase reactant) or intestinal protein losses due to inflammation [23, 61] *AST/ALT/Alkaline phosphatase/GGT:* Role in evaluating for extra-intestinal complications of inflammatory bowel disease [66–68]
Stool Cultures – *E. Coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* species	Infection	Evaluate for primary infectious colitis, which may mimic inflammatory bowel disease and exclude co-infection, which may complicate disease [114, 115]
Clostridium difficile PCR	Infection	Evaluate for primary infection and co-infection. In patients with inflammatory bowel disease, *C. difficile* is the most common infectious agent identified [10, 116]
Stool calprotectin	Elevation	Alternative inflammatory marker, which appears to be a direct measure of intestinal inflammation. Potential role in assessing disease activity and predicting relapse in patients with inflammatory bowel disease [126, 127, 130]
Stool lactoferrin	Elevation	Another inflammatory marker that demonstrated in preliminary studies the potential of being utilized as a measure of intestinal inflammation. As with calprotectin, has the potential role of assessing response to therapy [126, 127, 130]
IBD serologies	Positive ASCA (IgA or IgG), pANCA, anti-OmpC, anti-CBir	May aid in classifying disease subtype and play a role in therapeutic decisions (prognostic factor). Inadequate screening tool due to low sensitivity compared to clinical history and routine laboratory tests [1, 95, 100, 101]

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *IBD* inflammatory bowel disease, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* Gamma glutamyl transpeptidase, *ASCA* Anti-Saccharomyces cerevisiae (ASCA), *pANCA* perinuclear antinuclear cytoplasmic antibody, *OmpC* outer membrane protein

supplementation [17]. In one recent, prospective treatment adult trial, parenteral iron therapy appears to be more efficient than oral iron therapy [18]. Further, there are some reports that suggest that oral iron therapy affects the gut microflora in a manner counter-productive to successfully treating the iron deficiency compared to those receiving parenteral therapy [19].

The reported prevalence of anemia is variable in IBD, but anemia appears to be more prevalent in CD compared to UC [20]. In one population-based adult Scandinavian study from Denmark, Norway and Sweden, the overall prevalence of anemia in IBD was 19% with iron deficiency and anemia of chronic disease being the primary etiologies [20]. Additionally, anemia may be more common in children compared to adolescents and adults [21]. Using the WHO age-adjusted definitions of anemia, Goodhand et al. [21] assessed the prevalence, severity, type, and response to treatment of anemia in patients attending pediatric,

adolescent, and adult IBD clinics at a single center. These authors observed the prevalence of anemia to be 70% (41/59) in children, 42% (24/54) in adolescents, and 40% (49/124) in adults ($p < 0.01$). Overall, children (88% [36/41]) and adolescents (83% [20/24]) were more often iron-deficient than adults (55% [27/49]) ($p < 0.01$). In one study from Saudi Arabia, anemia was found in 86% of children affected by either ulcerative colitis or Crohn disease [22]. In other studies, anemia has been described occurring in 16–77% of patients with CD (16%, 58%, 70%, and 77% reported in pediatric cohorts) [14–17, 21, 23–26] and 9–67% of patients with ulcerative colitis (30% reported in one pediatric cohort) [17, 23, 26].

The cause of iron deficiency with or without frank anemia is likely multifactorial in both CD and UC [27]. In CD, anemia may result from iron, folate, or vitamin B12 micronutrient deficiencies from under or malnutrition which commonly accompanies extensive small bowel disease, particularly if the ileum is involved [27]. In addition, anemia may result from gross or occult gastrointestinal blood loss due to underlying intestinal inflammation. Finally, iron deficiency and/or anemia may be due to decreased overall iron stores due to chronic disease, and lack of appropriate dietary intake to replace iron stores [27]. The anemia observed in ulcerative colitis is generally the result of iron losses from chronic intestinal bleeding, but as with CD can be due to anemia of chronic disease. The assessment of iron status in IBD in many cases is rather difficult due to coexistent inflammation secondary to chronic disease [28]. For this assessment, several indices and markers have been suggested. Ferritin seems to play a central role in the definition and diagnosis of anemia in IBD and transferrin, transferrin saturation (Tsat), and soluble transferrin receptors have also been found to be useful markers in clinical practice. All these biochemical markers have limitations because they may be influenced by factors other than changes in iron balance. In addition, the iron metabolism regulators hepcidin and prohepcidin are still under investigation in IBD. Erythrocytes parameters like the red cell distribution width (RDW) and the percentage of hypochromic red cells as well as reticulocyte parameters such as hemoglobin concentration of reticulocytes, red blood cell size factor and reticulocyte distribution width could be useful markers for the evaluation of anemia.

Anemia of chronic disease that can be seen in IBD is also believed to be multifactorial in its etiopathogenesis. Three potential mechanisms leading to the anemia associated with chronic disease have been recently postulated, namely, (1) anemia results as a consequence of cytokine activation and subsequent alteration of iron homeostasis, (2) anemia occurs due to the inhibition of erythropoiesis, and (3) a shortened red blood cell half-life is associated with chronic disease and thereby results in the anemia [16, 29]. Additionally, the anemia of chronic disease such as that found in IBD involves erythropoiesis disturbance due to circulating inflammation mediators. In one study by Tsitsika et al., erythropoietin (Epo) levels in children and adolescents with IBD were investigated and correlated to disease activity [30]. In this particular study [30] 33 patients with IBD were evaluated (18 boys, 15 girls) ages 4–15 years (median 11 years). Patients were separated into two study groups related to their disease activity; those with active disease ($n = 21$), and those in remission ($n = 12$). Chronic disease-associated anemia was present only in patients with active disease, and, those patients also had a significantly higher possibility of low, altered Epo levels than expected compared with patients with inactive disease. Thus, it appears that impaired Epo production is another mechanism of anemia of chronic disease development.

Once the diagnosis of anemia is established, the etiology should be further investigated so treatment can be initiated. For macrocytic anemias, folate, vitamin B12, and methylmalonic acid levels should be obtained. Iron studies including ferritin, total iron binding content (TIBC), and iron levels should be evaluated in cases of microcytic anemia. However, the results of these studies may be difficult to interpret, as ferritin, a measure of iron stores, is also an acute phase reactant and may be falsely elevated in inflammatory conditions. Further in one recent retrospective pediatric study of 50 children with IBD compared to an equivalent number of celiac disease patients and controls demonstrated that serum hepcidin is increased in IBD children with active disease and it is responsible for iron malabsorption [31]. Thus, in patients with a microcytic anemia, obtaining a soluble transferrin receptor in addition to standard iron studies may be helpful in differentiating iron deficiency anemia and anemia of chronic disease [32–34]. Soluble transferrin receptor concentration, which is not affect by inflammation, is elevated in iron deficiency anemia, but remains normal in anemia of chronic disease [32–34]. In addition to soluble transferring receptor, intestinal ferroportin expression should be considered as a marker of anemia in relationship to inflammatory bowel disease and particularly Crohn disease in children. In a study performed by Burpee et al. [35], intestinal iron exporter ferroportin expression was studied in subjects with and without CD. In this investigation, the authors evaluated duodenal mucosal biopsies from 29 pediatric subjects, 19 of whom had CD and 10 were without CD. The authors observed that intestinal ferroportin protein was higher in anemic CD subjects than in nonanemic CD subjects, whereas ferroportin mRNA levels were not significantly different. Thus, intestinal ferroportin protein is upregulated in anemic CD subjects, suggesting yet another pathway for the iron deficiency and the anemia observed in children with CD [31, 35]. In a recent meta-analysis of studies comparing parenteral versus enteral iron therapy, IV iron demonstrated a higher efficacy in achieving a hemoglobin

rise of >/=2.0 g/dL as compared to oral iron (OR: 1.57, 95% CI: 1.13, 2.18). Treatment discontinuation rates, due to adverse events or intolerance, were lower in the IV iron groups (OR: 0.27, 95% CI: 0.13, 0.59). The authors concluded that IV iron appears to be more effective and better tolerated than oral iron for the treatment of IBD-associated anemia, likely related to some of the pathways of iron malabsorption described above [36].

Acute Phase Reactants: Platelets

In inflammatory conditions such as CD and UC, there is a rise in acute phase reactant proteins as a result of chemokine stimulation. The assessment of acute phase reactants has been employed as laboratory tests in the standard work-up of the child with suspected IBD, as well as other inflammatory conditions in pediatric patients (e.g., juvenile rheumatoid arthritis) [37, 38]. Reactive thrombocytosis, a nonspecific marker of inflammation, is a result of this acute phase response. Since the first published paper describing the association of thrombocytosis with chronic IBD by Morowitz et al. [39], the characterization of platelet elevation in the peripheral blood has been a "standard" part of the work-up of patients for suspected IBD, and in the monitoring of their disease activity. Some studies of the pathogenesis of IBD have implicated platelets in the propagation of intestinal inflammation. In a murine model of intestinal inflammation, CD40–CD40L appears to be involved in the pathogenesis of intestinal inflammation, and suggest that modulation of leukocyte and platelet recruitment by activated, CD40-positive endothelial cells in colonic venules may represent a major action of this signaling pathway. In addition, Kayo et al. [40] evaluated the role of platelets in inflammation in peripheral blood and in the mucosa of a cohort of patients with active UC. These investigators compared the group of patients with active UC to patients with inactive UC and a small cohort of healthy controls. The authors observed a close association between activated platelets and neutrophils in both the affected colonic mucosa and peripheral blood of patients with active-phase UC compared to the normal volunteers (i.e., healthy controls) and those with inactive UC. The investigators inferred from their study results that a platelet-neutrophil association may play a role in the progression of inflammatory processes in UC [40]. There is also evidence that coagulation activation may mediate and amplify inflammatory cascades in IBD, especially via activating proteinase-activated receptor related pathways [41]. Patients with CD and UC are at least three to fourfold increased risk of developing thromboembolic (TE) complications compared to control patients [41]. Although the etiology is multifactorial, thromboembolic phenomena in IBD is largely attributable to coagulation activation and platelet aggregation during systemic inflammation [41]. Thus, it appears that platelets may in fact play more of a role in the propagation of intestinal inflammation and potentially some of the severe sequelae (e.g., thromboembolic processes) of the system inflammation of IBD, rather than being a simple "biomarker" of IBD [37, 41].

In children referred for endoscopy for evaluation of abdominal pain, diarrhea, rectal bleeding, weight loss, or mouth ulcerations, 85% of patients with CD and 70% of patients with UC had elevated platelet counts compared to 6% of children with normal endoscopic assessment [23]. The presence of thrombocytosis may be overestimated in this study, or a unique response in the child with IBD as a lower prevalence of increased platelets in IBD is reported in adults [42–44]. However, an elevated platelet count in a child with chronic intestinal symptoms should raise clinical suspicion of underlying intestinal inflammation. In one study evaluating pediatric patients with chronic abdominal complaints, the presence of an abnormal hemoglobin and/or elevated platelet count on a routine CBC was able to differentiate between IBD and healthy controls, with 90.8% sensitivity and 80.0% specificity [45]. Furthermore, the platelet count may help differentiate between IBD and infectious processes, as thrombocytosis is a relatively uncommon finding in diarrhea associated with enteric pathogens [42].

Mean platelet volume (MPV) is influenced by the degree and type of mucosal and system inflammation. One study analyzed overall accuracy of MPV in disease activity and compared MPV with other inflammatory markers in 61 UC patients and 27 healthy subjects [46]. MPV was compared to ESR, CRP, and white blood cell count. The authors found that MPV accuracy was roughly equivalent to standard acute phase reactants and was significantly lower in UC patients and particularly active UC patients than controls [46]. Thus MPV may be another indicator of intestinal inflammation and a useful marker in patients with symptoms concerning for IBD.

Acute Phase Reactants: Erythrocyte Sedimentation Rate (ESR) and C-reactive Protein (CRP) and Other Markers

ESR and CRP are two other nonspecific measures of inflammation which should be included in the evaluation of patients with suspected IBD [47]. Both ESR and CRP have been investigated in IBD for a number of reasons, namely, (1) diagnostic and differential diagnostic purposes, (2) assessment of disease activity (i.e., PDCAI) and risk of complications, (3) prediction of CD or UC relapse, and (4) for monitoring the effect of therapy. 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, tumor necrosis factor α, and IL-1β – all proinflammatory chemokines which are present in active IBD in both children and adults. CRP has a relatively 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 it is still up for question, overall, CRP may be a better measure for assessing disease activity and predicting relapse. In CD in particular, CRP appears to correlates well with disease activity, and thus is one objective marker that may be helpful in distinguishing IBD from noninflammatory conditions [48]. Additionally, in clinical trials with biological therapies, elevated CRP levels prior to initiation of therapy are associated with higher response rate, whereas normal CRP levels are predictive of higher placebo response rates [48]. However, despite the advantages of CRP over other markers, it is still far from ideal. Not all IBD patients, CD or UC, mount a CRP response, 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 as compared to transmural disease differences among UC and CD, or whether this acute inflammatory marker elevation is genetically driven.

Both ESR and CRP can be elevated to varying degrees in IBD and therefore are helpful in distinguishing inflammatory from functional disorders. In a study of 91 children referred for chronic gastrointestinal symptoms the CRP was elevated in 100% of patients with CD and 60% UC, and ESR was elevated in 85% of patients with CD and 23% of patients with UC [23]. None of the patients with polyps or normal investigations had elevation of either marker. In adults with chronic abdominal symptoms, all patients with CD and 50% of patients with UC had elevated ESR and CRP, whereas none of the patients with functional disorders had elevation of both markers [49]. Therefore, using these markers in combination may increase the diagnostic yield [50].

Overall, the response of ESR and, in particular, CRP in UC appears to be less robust, with elevated values found in more extensive colitis compared to limited disease [51–54]. However, the development of highly sensitive CRP assays may improve the sensitivity of this test, even in patients with limited disease [55]. In a study by Poullis et al. [55], the authors evaluated 224 adult patients and determined the accuracy of the CRP in distinguishing IBD from functional GI disease. Using a newly developed enzyme-linked immunoassay approach to CRP measurement, the authors determined that a CRP cutoff value of 2.3 mg/L had a sensitivity of 100% and a specificity of 67% in differentiating functional bowel disease from new cases of IBD [55]. Compared to ESR, CRP has a shorter half-life and thus returns to baseline values more rapidly once the inflammatory stimulus has resolved. Because of this rapid decline, CRP may be a better measure of remission and response to therapy than other inflammatory markers in patients with IBD [48].

Other laboratory markers, including leukocyte and platelet count, albumin, and 1-acid glycoprotein (orosomucoid), have been studied either less extensively in IBD, particularly in pediatric populations, or, have proven to be less useful than more traditional biomarkers such as CRP [48]. In a small cohort-sized study of adult UC patients ($N = 28$) before and after 8 week therapy for example, fecal samples were analyzed for myeloperoxidase (MPO), eosinophil protein X (EPX), mast cell tryptase, IL-1beta, and TNF-alpha using immunoassays [56]. Blood samples were analyzed for MPO, EPX, C-reactive protein, orosomucoid, and leucocyte counts. The investigators determined that fecal MPO and IL-1beta levels were elevated in all patients at inclusion despite different disease phenotypes (i.e., extent of disease). Striking reductions in fecal levels of MPO, EPX, tryptase, and IL-1beta were observed after 4 weeks of treatment in 20/28 patients [56]. Levels of fecal markers correlated with endoscopic scores, histological severity and circulating blood acute phase reactants; i.e., orosomucoid [56]. In one small study of Scandinavian adults with Crohn disease undergoing infliximab therapy, Crohn Disease Activity Index, the Harvey Bradshaw Index, C-reactive protein, as well as orosomucoid and albumin reached normal levels during infliximab treatment [57]. Orosomucoid was as sensitive as the more "traditional" inflammatory markers and correlated tightly with physician global assessment and CDAI [57]. Clearly as we learn more about the pathogenetics of IBD, CD, and UC, these types of novel biomarkers and others to be developed can serve as noninvasive, objective biomarkers for the diagnosis and monitoring of IBD.

Other Laboratory Evaluations

Liver function tests and electrolyte panels may add additional information to aid the clinician in differentiating IBD from non-IBD, in the determination of the IBD phenotype and, in particular, the presence or absence of extra-intestinal manifestations such as liver disease [58, 59]. Although severe liver disease can be the first presentation of IBD in pediatric patients, hypoalbuminemia, which may be due to liver parenchymal damage, decreased production and/or due to bowel injury accompanied by increased fecal loss, is a more frequent finding at diagnosis [59]. Hypoalbuminemia is observed in both CD and UC; however, overall decreased serum albumin appears to be present at a much higher frequency in CD. In pediatric cohorts, hypoalbuminemia has been reported in 35–64% of patients with CD and 15% of patients with UC [23, 24, 60–64]. In a relatively small-sized ($N = 57$) pediatric study of children with UC from Saudi Arabia, hypoalbuminemia was observed in over half (i.e.,

54%) of the cohort evaluated, with disease severity correlating with the degree of hypoalbuminemia [22]. In addition to being useful in the diagnosis of IBD compared to non-IBD, as well as a factor in the assessment of the child's overall nutritional status, hypoalbuminemia when present, may have value as a prognostic factor for surgical risk [60] as well as for osteopenia and decreased bone mineral density scores [62]. Albumin can also be used as a marker for response to therapy. In an adult multicenter clinical trial evaluating one of the biologics for therapy of CD, the authors investigated the effect of adalimumab on changes in laboratory values using data from CHARM trial [65]. In a total of 778 adult patients, adalimumab every-other-week ($N = 260$), adalimumab weekly ($N = 257$), or placebo ($N = 261$), the authors observed significant improvements in nutritional, hematologic, and inflammatory markers, including and specifically albumin, in moderately to severely active CD [65].

Similar to the pathobiology of anemia associated with IBD, the etiology of hypoalbuminemia in the child or adolescent with IBD is multifactorial, with protein loss from intestinal inflammation, decreased albumin production (negative acute phase response), and long-term poor nutrition all contributing to the overall low circulating levels of this important protein [52, 61, 63].

Elevation of AST and ALT may also be present on this initial screen in the evaluation of a patient with suspected IBD. In one study by Mendes et al. [66], the prevalence of abnormal hepatic biochemistries and chronic liver disease in a cohort of IBD patients was described in a retrospective case-control fashion. Patients with normal and abnormal liver biochemistries were compared, and in the cohort of 544 patients, abnormal hepatic biochemistries were present in nearly one-third of these adult patients. Contrary to what the investigators hypothesized, abnormal liver biochemistries in this single center cohort were not associated with IBD activity. These authors recommended that persistently abnormal hepatic biochemistries should be evaluated, but to use caution and not immediately attribute these abnormal liver biochemistries to IBD activity [66]. Abnormal liver biochemistries may also be primarily related to poor nutrition as a result of active disease, and thus spontaneous resolution of these transient elevations are common [67].

However, when AST/ALT are persistently elevated or seen in association with an elevated alkaline phosphatase, elevated direct bilirubin and/or γ-glutamyl transpeptidase, the extra-intestinal complication of primary sclerosing cholangitis (PSC) or autoimmune hepatitis/overlap syndrome should be considered. PSC is reported complication in 3–15% of children with IBD and can precede or occur coincident with diagnosis of IBD [68–71]. In a U.S. population-based health maintenance organization study, the prevalence of PSC in conjunction with IBD was characterized in addition to the demographic differences between racial/ethnic groups in patients with PSC compared to non-IBD and non-liver disease controls. Using the Northern California Kaiser Permanente (KP) database, the authors identified 169 (101 males) cases fulfilling PSC diagnostic criteria with a mean age at diagnosis of 44 years (range 11–81); age-adjusted point prevalence was 4.15 per 100,000 on December 31, 2005 [72]. IBD was present in 64.5% (109/169) cases and was significantly more frequent in men than women with PSC (73.3% and 51.5%, respectively, $p = 0.005$) [72]. In another small-sized single center study ($N = 29$), the incidence of IBD in PSC patients was 68.9% (20/29) [73]. The investigators showed two peaks in the age distribution of PSC with male PSC patients demonstrated a first peak and female patients a second peak. Male PSC-IBD patients were in their teens and 20s making the first peak and female PSC-IBD patients were in their 50s and 60s making the second peak. Of note, the study demonstrated that PSC-IBD patents were significantly younger than the patients without IBD (33.6 vs. 58.9 years, $p < 0.001$) [73]. With regards to pediatric patients, Wilschanski et al. [70] demonstrated of 32 children with PSC, the majority were diagnosed in their second decade (median age: 13 years) and four children presented before the age of 2 years. Seventeen of the 32 patients had inflammatory bowel disease (IBD), all with colitis; 14 UC, and 3 CD [70]. Eight patients presented with chronic liver disease before clinical onset of IBD. Thus, of the hepatic pathologies reported associated with IBD in children and adults, PSC remains the more common presentation. In one longitudinal, cohort study by Feldstein et al. [68] 52 children with cholangiography-proven PSC were followed to determine the long-term outcome (mean follow-up was 16.7 years) of children with PSC diagnosed over a 20 year period (34 boys and 18 girls; mean age 13.8 ± 4.2 years; range, 1.5–19.6 years). Two thirds presented with symptoms and/or signs of PSC and 81% had concomitant IBD [68]. During follow-up, 11 children underwent liver transplantation for end-stage PSC and 1 child died with the median (50%) survival free of liver transplantation being 12.7 years. Compared with an age- and gender-matched U.S. population, survival was significantly shorter in children with PSC ($p < 0.001$). Using a statistical regression model for analysis, the authors determined that lower platelet count, splenomegaly, and older age were associated with shorter survival. Moreover, presence of autoimmune hepatitis overlapping with PSC ($p = 0.2$) or medical therapy ($p = 0.2$) did not affect survival. Thus, the authors concluded that PSC, whether associated with IBD or not, significantly decreases survival in this child population [68]. Furthermore, genotype–phenotype studies recently demonstrated that a continuum of disorders exists within inflammatory bowel disease, much better explained by placing patients into three groups (ileal Crohn disease, colonic Crohn disease, and ulcerative colitis) rather than simply by Crohn disease and ulcerative colitis as currently

defined. The authors further observed that within these phenotypes "risk" can be determined with respect to those more likely to develop extra-intestinal manifestations such as PSC [74].

Renal as well as pancreatic disease may also be important extra-intestinal manifestations of IBD or can be adverse events associated with IBD pharmacotherapy [75–80]. In a multicenter study from Israel, both adults and children presenting with acute pancreatitis as the first symptom of IBD were retrospectively identified (10 years, 7 university hospitals) [80]. These authors demonstrated that 10 of 460 pediatric patients with IBD (2.17%), compared with only 2 in 3500 adults (0.06%) presented with pancreatitis. Eight children had colonic disease (four Crohn disease, four ulcerative colitis [three pancolitis]) with the mean amylase level being 1419 (range 100–1370) and three children (30%) having mildly elevated transaminases [80]. It is important to note was that median time between onset of the first episode of acute pancreatitis in relation to onset of IBD was 24 weeks (range 1–156) and the most common presentation in this cohort was abdominal pain.

Similarly, renal disease may precede diagnosis of IBD. Although small in sample size, Izzedine et al. [81] described four patients with severe interstitial nephritis demonstrated on histopathological examination of kidney biopsy specimens. Renal failure was discovered before or simultaneously with the diagnosis of CD, and patients were not treated with mesalamine. More importantly, impairment of renal function progressed to end-stage renal failure in three of the four patients [81]. A similar small case series of two pediatric patients with renal disease occurring concurrently with diagnosis of IBD has been reported [82]. Thus, with respect to appropriate adjunct or complementary lab tests to obtain in the work-up of a child with suspected IBD, given the reports of interstitial nephritis in patients with Crohn disease in the absence of 5-aminosalicylate exposure, a baseline comprehensive chemistry panel should be considered during the initial evaluation. Moreover, amylase and lipase should be considered at some point in the initial evaluation, during phenotyping once the diagnosis has been made, and in particular, where clinical signs and symptoms raise suspicion of pancreatic disease; prior to or after initiation of therapy particularly those medications with a predilection (e.g., 6MP, 5-ASA) for pancreatitis as a side effect.

The above paragraphs highlight the standard evaluation that is recommended for all children with history and physical exam findings suspicious for IBD. These diagnostic tests may aid the clinician in the differentiation of UC and CD from functional bowel disorders and infectious etiologies. However, because the clinical presentation of IBD is so diverse and symptoms can be nonspecific, at times, it may be difficult to distinguish between inflammatory and functional disorders. In fact, since May 13, 1932, when Dr. Crohn and his colleagues, Oppenheimer and Ginzburg, presented a paper on terminal ileitis describing the features of Crohn disease to the American Medical Association, the average time from onset of symptoms to definitive diagnosis continues to be prolonged, ranging from 6 to 18 months [83–85].

Several other noninvasive studies have been proposed to aid in the diagnosis of inflammatory bowel disease including IBD serologies, fecal calprotectin, and lactoferrin. The following section reviews these tests including a brief overview of the use of IBD serology and the evidence to support or disprove their use in the preliminary evaluation of the child with suspected IBD. In addition, this section will describe the stool tests which are an essential part of the initial work-up of the child with suspected IBD, and includes a discussion of more novel markers of intestinal inflammation, fecal calprotectin, and fecal lactoferrin.

Specific Blood Tests: Inflammatory Bowel Disease Serologies

Anti-*Saccharomyces cerevisiae* (ASCA), an antibody response against *Saccharomyces cerevisiae* and perinuclear antinuclear cytoplasmic antibody (pANCA), an antibody response toward nuclear antigens with a perinuclear pattern, are two immunologic markers detected in IBD. There is much debate in both the pediatric and adult clinical settings regarding the proper use of these serologies in the evaluation of IBD, and there have been several studies assessing the accuracy and clinical utility of ASCA and pANCA in children with IBD [1, 5, 86–95]. Although these investigations differ in their study design and in some cases the type of serological profile obtained, overall, these markers appear to be reasonably specific for both CD and UC. In the reported studies, ASCA (IgG or IgA) specificity ranged from 88% to 97% for CD [88, 90–93] and pANCA specificity ranged from 65–95% for UC [87, 88, 90–93]. In children, the specificity of the combined serologies in differentiating IBD from non-IBD has been reported to range from 84% to 95% [1, 5, 88, 90, 94]. Unfortunately, the sensitivity of these serologies has been shown to be poor with overall sensitivity ranges reported between 55% and 78% [1, 5, 86, 88, 90, 94]. A meta-analysis of 60 adult and pediatric studies yielded similar findings and reported the sensitivity and specificity of ASCA IgG or IgA positive and pANCA negative for the detection of Crohn disease as 55% and 93%, respectively [96]. The sensitivity and specificity of positive pANCA for detection of UC were lower at 55.3% and 92.8%, respectively [96]. Therefore, a negative test result does not exclude the diagnosis of IBD, particularly in those patients with nonspecific symptoms such as abdominal pain and intermittent diarrhea. The addition of anti-OmpC, an antibody to the

outer membrane porin of *Escherichia coli*, appears to add little to the diagnostic accuracy of this serologic panel in children [92, 93]. In two pediatric studies, the overall sensitivity of anti-OmpC for both CD and UC was very low [92, 93]. However, the use of the additional IBD serologies may help identify a small number of IBD patients who had negative ASCA and pANCA [92, 93, 97]. Younger children appear to have the greatest proportion of seronegativity to ASCA and ANCA and therefore these additional markers, particularly anti-cBir, may be most helpful in this population [97]. Moreover, with an increasing number of candidate genes being identified in patients with IBD, particularly CD, other serological markers have been identified that may increase the overall sensitivity of the assays [98]. For example, patients carrying the NOD2 mutations have an increased adaptive immune response to commensal organisms as measured by higher titers of antimicrobial antibodies, such as anti-CBir and ASCA [98]. Thus, use of a combination of serologic, genetic and inflammatory markers may further improve the diagnostic accuracy and utility of these tests for discriminating IBD from noninflammatory conditions [99].

Although their specificity is reasonable, overall ASCA and pANCA appear to be less sensitive than clinical history and routine laboratory tests (hemoglobin and ESR) in the evaluation of pediatric IBD. In a retrospective study, Khan et al. [94] evaluated 177 pediatric subjects who had pANCA and ASCA, hemoglobin, ESR and colonoscopy as part of their initial evaluation. In this study, 90 patients were diagnosed with IBD, and of those, 52 had UC and 39 were diagnosed with CD. Combining abnormal hemoglobin and/or ESR with rectal bleeding, the most distinguishing symptom for IBD in this study cohort, was more sensitive than positive ASCA and/or pANCA (86% versus 68%) and identified 86% of patients with IBD prior to endoscopy. A study by Sabery et al. [1] yielded similar findings. In this retrospective study which included 210 pediatric subjects, 40 with IBD, the sensitivity of ASCA and pANCA was again compared to hemoglobin and ESR [1]. The presence of an abnormal hemoglobin or ESR was the more sensitive screen, with a sensitivity of 83%, compared to 73% for the First Step® modified assay (Prometheus laboratories, San Diego, CA), and 60% for the confirmatory panel, which included anti-OmpC. In the subset of patients without rectal bleeding, a group whose symptoms may be more difficult to differentiate from functional disorders, the sensitivity of ASCA and pANCA decreased to 55% whereas the sensitivity of an abnormal hemoglobin or ESR remained high at 91%. In pediatric patients, the addition of antibodies to cBir flagellin to the serological panel does not appear to improve the diagnostic yield of this panel. A retrospective study of 304 pediatric patients with suspected IBD reported a sensitivity of 67% and specificity of 76% of the combined serological panel, and for anti-cBir specifically, the sensitivity and specificity were 50% and 53%, respectively [95]. As mentioned, combination of standard laboratory tests (hemoglobin, platelet count and ESR) had higher predictive value, with sensitivity of 72%, specificity of 94% and positive predictive value of 85% [95]. Additionally, as hemoglobin and ESR are both components of the PCDAI, they have added value as markers of disease severity and clinical response.

Given the cost of these tests and overall poor sensitivities documented in several pediatric studies, particularly compared to other clinical and laboratory parameters, currently, serology testing does not appear to have additive value as a screening test in the initial diagnostic work-up for patients with suspected IBD. However, these serologies may have a role in predicting disease course and identifying patients at risk for complicated disease. In a study by Targan et al. [100] 484 sera previously employed for a study evaluating other serological markers of IBD (namely, ASCA, pANCA, OmpC) were tested for anti-CBir1 by enzyme-linked immunosorbent assay. Interestingly, the authors observed that the presence and level of immunoglobulin G anti-CBir1 were associated with CD independently and were associated with a unique phenotype of CD, namely, small-bowel, internal-penetrating, and fibrostenosing disease. Papadakis et al. [101], also demonstrated that anti-CBir1 serum reactivity in CD patients is independently associated with fibrostenosing disease and complicated small bowel CD. Anti-OmpC and anti-IL2 have also been shown to be associated with more aggressive disease course in adult patients with CD [102]. As a single marker, ASCA may be most predictive of aggressive disease and several studies have demonstrated ASCA positivity (IgG or IgA) alone was associated with complicated disease behavior, perianal disease, and risk for surgery in both pediatric and adult cohorts [92, 103–105]. In children with CD, the presence of multiple serologic markers and degree of antibody elevation has been associated with more severe disease phenotypes, with frequency of internal-penetrating and fibrostenosing disease increasing with the number of antibodies present [106, 107]. Similar to adult data, anti-Omp C and anti-IL2 were independently associated with these complications [106]. A more recent cross-sectional study of adults with CD suggests that in addition to quantitative serologic markers, the presence of NOD2 genetic variants is associated with complicated disease [108]. Overall the data for pANCA and disease stratification/course is less robust, and a recent study demonstrated no correlation between disease severity and pANCA titers [109]; however, pANCA reactivity may be associated with primary nonresponse to anti-TNF therapy pediatric patients and absence of this marker may help predict long-term response to this medication [110, 111].

Approximately 10% of patients with IBD are diagnosed with IBD-unclassified (IBD-U), and this diagnosis may be higher in younger children as isolated colonic CD is more

common [97]. There is interest in using these serologies to classify disease subtype in children with IBD-U and to assist in therapeutic decisions such as colectomy. In one longitudinal study of 406 children with Crohn's colitis, UC and IBD-U, ASCA + differentiated well between Crohn's colitis, IBD-U and UC (specificity 83%, PPV 96%); pANCA + had similar positive predictive value, but much lower sensitivity and specificity (65% and 66%, respectively) [112]. However, as the most common serologic profile in IBD-U is ASCA−/pANCA−, serology overall has lower utility in predicting subsequent disease type [112, 113]. Therefore, based on the above data, perhaps for now, the use of these serologies should be reserved as a potential prognosticator of disease course and assessment for risk for complicating disease.

Stool Evaluation

The presentation of pediatric inflammatory bowel disease can be markedly variable. However, those children who present with "classic" gastrointestinal complaints such as diarrhea and abdominal pain should have a thorough stool evaluation to rule out bacterial and parasitic etiologies of these symptoms. Standard stool cultures to look for enterohemorrhagic *Escherichia. coli, Salmonella, Shigella, Yersinia*, and *Campylobacter* species, *Clostridium difficile* assay, preferably by PCR, and ova and parasite studies to look for *Entameoba histolytica* and other parasites are a necessary part of the work-up to differentiate infectious versus inflammatory enterocolitis and should be obtained prior to invasive procedures. In particular, *Yersinia enterocolitica* infections may mimic CD and thus specific emphasis should be placed on looking for this organism as isolation can be increased by using selective media [114, 115]. Also, defects in mucosal barrier function can predispose patients with IBD to infectious colitis, and *Clostridium difficile (C. difficile)* is the most common infectious agent identified [10, 116]. Overall *C. difficile* infection has been a growing problem and the rates of *C. difficile* infection have been increasing as have pediatric hospitalization due to this infection [117]. Clinical symptoms of *C. difficile* and IBD are similar and the prevalence of *C. difficile* is significantly greater in pediatric patients with IBD compared to children without this diagnosis [118, 119]. A positive stool test therefore does not rule out the possibility of IBD, and thus patients with a suspicious clinical history who do not improve with appropriate treatment of stool pathogens should have further diagnostic evaluations. In addition to differentiating between infectious colitis and IBD, there has been a lot of recent attention towards infectious agents in the etiopathogenesis of IBD; with focus either being on enteric microflora (i.e., commensals) as compared to infecting pathogens in the genetically susceptible host [4, 120].

Fecal Calprotectin

Calprotectin, a calcium binding protein in the S100 family, is an abundant protein in neutrophils, and to a lesser extent, macrophages and monocytes, accounting for approximately 60% of the cytosolic protein in neutrophils [121–123]. Calprotectin has bacterostatic and antifungal properties, and thus likely contributes to neutrophilic defenses [124]. In healthy individuals, concentrations of calprotectin are approximately six times higher in stool than plasma [123]. In IBD, a spot fecal calprotectin level correlates well with fecal excretion of [111] indium white cells, suggesting this protein can be an alternative marker of intestinal inflammation [125, 126]. Fecal calprotectin is easy to measure, resistant to proteolysis and stable in stool for 7 days, and thus has been proposed as a simple noninvasive investigative tool, which may help distinguish inflammatory from functional disorders [47, 123, 127–129].

Several studies have shown elevated fecal calprotectin levels in patients with both UC and CD compared to healthy controls and patients with irritable bowel syndrome (IBS) [47, 127–129]. In one large study of 602 new patient referrals who had symptoms compatible with either irritable bowel syndrome or organic disease, including 189 patients later diagnosed with IBD, fecal calprotectin levels of >10 mg/L had a sensitivity of 89% and specificity of 79% for organic diseases [130]. This test was more sensitive than either ESR or CRP and an abnormal fecal calprotectin had an odds ratio for disease of 27.8 [130]. A similar, but small cohort-sized study by Carroccio et al. [131] using the newer fecal calprotectin assay yielded a somewhat lower sensitivity for organic disease (sensitivity 66%), but similar specificity (84%). However, in the small subset of 9 adult patients with inflammatory bowel disease, the sensitivity and specificity of fecal calprotectin was 100% and 95%, respectively [131]. A subsequent meta-analysis of six prospective adult studies that assessed the diagnostic accuracy of fecal calprotectin in patients with suspected IBD revealed a pooled sensitivity and specificity of 93% and 96%, respectively [132]. A more recent meta-analysis of 8 prospective studies with combined 1062 patients found that patients with a fecal calprotectin level of </ = 40 μg/g had ≤1% chance of having IBD; however, the positive predictive value of this test was lower and a high calprotectin could not completely exclude IBS [133]. Other studies have demonstrated that fecal calprotectin may be superior to CRP in discriminating between IBD and irritable bowel syndrome with a diagnostic accuracy of 80–89% compared to 64–73% for CRP [134, 135].

There have also been several studies evaluating fecal calprotectin in the pediatric population. Carroccio et al. [131] study cohort included 50 children with chronic diarrhea, and the assay had a higher sensitivity (70%) and specificity (93%) in pediatric patients than in adults. Some pediatric studies have reported even higher sensitivity of the fecal

calprotectin assay. Fagerberg et al. [127] obtained fecal calprotectin levels in 36 pediatric patients with gastrointestinal symptoms who underwent colonoscopy for suspected inflammation. Using the standard upper reference limit of <50 μg/g for the modified assay, the test has a sensitivity and specificity for inflammation of 95% and 93%, respectively. Using the older assay, Bunn et al. [136] reported a sensitivity of 90% and specificity of 100% for identifying intestinal inflammation in 36 pediatric patients who underwent either colonoscopy or ^{99}Tc-labeled white blood scans for suspected inflammatory bowel disease. As there was a strong suspicion of IBD in these studies, there may be some selection bias, which resulted in these higher sensitivities and specificities. Other pediatric studies have reported similar sensitivities but lower specificities of the fecal calprotectin assay in differentiating IBD from other conditions [137, 138]. Two meta-analyses of prospective pediatric studies revealed a pooled sensitivity and pooled specificity of 92–97% and 70–76%, respectively [132, 139], whereas meta-analyses that also included respective pediatric case-control studies, which may introduce more bias, reported slightly lower pooled specificities (65–68%), with similar sensitivities [50, 140]. With relation to CD, disease location (small bowel versus colonic involvement) does not appear to limit the utility of this test [141, 142]. Based on these collective results, it appears fecal calprotectin correlates well with the presence of histologic inflammation in pediatric patients. In patients where symptoms overlap with both IBD and IBS, obtaining fecal calprotectin testing prior to endoscopy may be a cost effective screening strategy, particularly when the suspicion of IBD is low [143].

Fecal calprotectin may offer some insight into the severity of inflammation in children with IBD, with levels correlating with severity of mucosal disease, with a correlation superior to clinical activity indexes and CRP [141, 142, 144, 145]. As it correlates with mucosal disease, fecal calprotectin may be surrogate for mucosal healing. In one small prospective study of 24 newly diagnosed children with CD, a drop in fecal calprotectin of >50% after therapy had a specificity of 82% for predicting inactive endoscopic disease [146]. In adults, a level ≤250 μg/g predicted endoscopic remission in CD with 94.1% sensitivity and 62.2% specificity, whereas in UC a level >250 predicted active mucosal disease with a sensitivity of 71% and specificity of 100% [147]. Additionally, there have been several studies evaluating fecal calprotectin's role in predicting disease relapse. One prospective study of 32 children with IBD found that 90% of patients with fecal calprotectin > 400 μg/g experienced clinical relapse whereas 89% with fecal calprotectin below this threshold remained in clinical remission [148]. A larger prospective multicenter adult study also demonstrated that calprotectin concentrations in patients who relapsed where higher than those who did not, with a fecal calprotectin level of >150 μg/g having a sensitivity of 69% and specificity of 69% to predict relapse [149]. Therefore, the assay offers an advantage over other nonspecific inflammatory markers as is appears to be a direct measure of intestinal inflammation and consequently may be followed prospectively in patients as a marker of disease activity and relapse. Although larger prospective pediatric clinical studies need to be performed, fecal calprotectin continues to offer promise in the evaluation of patients with suspected IBD and for monitoring disease activity prospectively.

Fecal Lactoferrin

Another potentially useful stool marker in patients with IBD is fecal lactoferrin. As with calprotectin, based on adult data, lactoferrin appears to be superior to CRP in differentiating between IBD and irritable bowel syndrome [134, 135]. In one adult study, this protein was shown to be the most useful of neutrophil-derived proteins in stool as a marker of intestinal inflammation [150]. In a large pediatric study in 148 children with CD, UC, irritable bowel syndrome, and healthy volunteers, fecal lactoferrin was shown to be a useful marker of inflammation in diagnosis and interval assessment, and it correlated well with the clinical activity indices and ESR [151]. Pfefferkorn et al. [152] similarly found fecal lactoferrin levels could be used to distinguish pediatric CD from non-IBD conditions, with sensitivity of 100%, specificity of 43% and negative predictive value of 100%. At higher cutoff values (≥60 μg/g), this marker could also be used to differentiate active from inactive disease with 84% sensitivity and 74% specificity [152]. In a meta-analysis of seven studies (n = 1012), four which included pediatric patients, fecal lactoferrin had a pooled sensitivity and specificity of 78% and 94%, respectively, in differentiating IBD from IBS [153]. Thus fecal lactoferrin appears to be another promising non-invasive marker of intestinal inflammation, and therefore a consideration in evaluation of patients with suspected inflammatory bowel disease. Similar to calprotectin, lactoferrin may also be useful to monitor response to therapy and predict clinical relapse [149, 154].

Summary

In the preceding paragraphs, we attempted to provide an overview of the laboratory tests, both blood and stool studies, available that can be used in the initial work-up of the child with suspected inflammatory bowel disease. Although a thorough clinical history and physical exam can raise suspicion of CD or UC it is important to include a focused laboratory evaluation. A combination of blood and stool tests may further differentiate between IBD and non-IBD in

particular, inflammatory disease, compared to infectious processes and functional bowel disorders. Not only can a carefully chosen combination of blood and stool studies help determine which child may require more invasive testing, but they can also be used in the initial phenotyping of the disease, i.e., CD versus UC. Moreover, there are laboratory tests available, specifically IBD serologic markers such as ASCA and anti-CBir1, which can be employed to subtype CD and potentially provide the clinician with the ability to prognosticate disease severity. The definitive diagnosis of IBD is made by combining historical features, physical examination, radiological findings, and endoscopy and biopsy. However, laboratory investigations provide important information about inflammation and function of other organ systems that may or may not be involved in the child with IBD, which ultimately helps guide the clinician toward more invasive testing, making a definitive diagnosis and even phenotyping the IBD that facilitates the ability for the clinician to employ more precise targeted optimal therapies.

References

1. Sabery N, Bass D. Use of serologic markers as a screening tool in inflammatory bowel disease compared with elevated erythrocyte sedimentation rate and anemia. Pediatrics. 2007;119:e193–9.
2. Hait E, Bousvaros A, Grand R. Pediatric inflammatory bowel disease: what children can teach adults. Inflamm Bowel Dis. 2005;11:519–27.
3. Auvin S, Molinie F, Gower-Rousseau C, et al. Incidence, clinical presentation and location at diagnosis of pediatric inflammatory bowel disease: a prospective population-based study in northern France (1988-1999). J Pediatr Gastroenterol Nutr. 2005;41:49–55.
4. Oliva-Hemker M, Fiocchi C. Etiopathogenesis of inflammatory bowel disease: the importance of the pediatric perspective. Inflamm Bowel Dis. 2002;8:112–28.
5. Dubinsky MC, Ofman JJ, Urman M, Targan SR, Seidman EG. Clinical utility of serodiagnostic testing in suspected pediatric inflammatory bowel disease. Am J Gastroenterol. 2001;96:758–65.
6. 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;41:416–21.
7. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology. 2011;140:1817–26 e2.
8. Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a pediatric Crohn's disease activity index. J Pediatr Gastroenterol Nutr. 1991;12:439–47.
9. Griffiths AM, Otley AR, Hyams J, et al. A review of activity indices and end points for clinical trials in children with Crohn's disease. Inflamm Bowel Dis. 2005;11:185–96.
10. Mylonaki M, Langmead L, Pantes A, Johnson F, Rampton DS. Enteric infection in relapse of inflammatory bowel disease: importance of microbiological examination of stool. Eur J Gastroenterol Hepatol. 2004;16:775–8.
11. Roy CN, Weinstein DA, Andrews NC. 2002 E. Mead Johnson Award for Research in Pediatrics Lecture: the molecular biology of the anemia of chronic disease: a hypothesis. Pediatr Res. 2003;53:507–12.
12. Koutroubakis IE, Karmiris K, Kouroumalis EA. Treatment of anaemia in inflammatory bowel disease. Aliment Pharmacol Ther. 2006;23:1273–4. author reply 4-5
13. Wells CW, Lewis S, Barton JR, Corbett S. Effects of changes in hemoglobin level on quality of life and cognitive function in inflammatory bowel disease patients. Inflamm Bowel Dis. 2006;12:123–30.
14. Thayu M, Leonard MG, RN B, Mamula P. Prevalence of anemia in incident pediatric Crohn disease. J Pediatr Gastroenterol Nutr. 2005;41:547.
15. Thayu M, Mamula P. Treatment of iron deficiency anemia in pediatric inflammatory bowel disease. Curr Treat Options Gastroenterol. 2005;8:411–7.
16. Gasche C, Lomer MC, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. Gut. 2004;53:1190–7.
17. Wilson A, Reyes E, Ofman J. Prevalence and outcomes of anemia in inflammatory bowel disease: a systematic review of the literature. Am J Med. 2004;116(Suppl 7A):44S–9S.
18. Han YM, Yoon H, Shin CM, et al. Comparison of the efficacies of parenteral iron sucrose and oral iron sulfate for anemic patients with inflammatory bowel disease in Korea. Gut Liver. 2016;10:562–8.
19. Lee T, Clavel T, Smirnov K, et al. Oral versus intravenous iron replacement therapy distinctly alters the gut microbiota and metabolome in patients with IBD. Gut. 2016.
20. Bager P, Befrits R, Wikman O, et al. The prevalence of anemia and iron deficiency in IBD outpatients in Scandinavia. Scand J Gastroenterol. 2011;46:304–9.
21. Goodhand JR, Kamperidis N, Rao A, et al. Prevalence and management of anemia in children, adolescents, and adults with inflammatory bowel disease. Inflamm Bowel Dis. 2012;18:513–9.
22. Saadah OI. Ulcerative colitis in children and adolescents from the Western Region of Saudi Arabia. Saudi Med J. 2011;32:943–7.
23. Beattie RM, Walker-Smith JA, Murch SH. Indications for investigation of chronic gastrointestinal symptoms. Arch Dis Child. 1995;73:354–5.
24. Burbige EJ, Huang SH, Bayless TM. Clinical manifestations of Crohn's disease in children and adolescents. Pediatrics. 1975;55:866–71.
25. Dyer NH, Child JA, Mollin DL, Dawson AM. Anaemia in Crohn's disease. Q J Med. 1972;41:419–36.
26. Thomson AB, Brust R, Ali MA, Mant MJ, Valberg LS. Iron deficiency in inflammatory bowel disease. Diagnostic efficacy of serum ferritin. Am J Dig Dis. 1978;23:705–9.
27. Cronin CC, Shanahan F. Anemia in patients with chronic inflammatory bowel disease. Am J Gastroenterol. 2001;96:2296–8.
28. Murawska N, Fabisiak A, Fichna J. Anemia of chronic disease and iron deficiency anemia in inflammatory bowel diseases: pathophysiology, diagnosis, and treatment. Inflamm Bowel Dis. 2016;22:1198–208.
29. Oustamanolakis P, Koutroubakis IE, Kouroumalis EA. Diagnosing anemia in inflammatory bowel disease: beyond the established markers. J Crohns Colitis. 2011;5:381–91.
30. Tsitsika A, Stamoulakatou A, Kafritsa Y, et al. Erythropoietin levels in children and adolescents with inflammatory bowel disease. J Pediatr Hematol Oncol. 2005;27:93–6.
31. Martinelli M, Strisciuglio C, Alessandrella A, et al. Serum hepcidin and iron absorption in paediatric inflammatory bowel disease. J Crohns Colitis. 2016;10:566–74.
32. Margetic S, Topic E, Ruzic DF, Kvaternik M. Soluble transferrin receptor and transferrin receptor-ferritin index in iron deficiency anemia and anemia in rheumatoid arthritis. Clin Chem Lab Med. 2005;43:326–31.
33. Markovic M, Majkic-Singh N, Subota V. Usefulness of soluble transferrin receptor and ferritin in iron deficiency and chronic disease. Scand J Clin Lab Invest. 2005;65:571–6.

34. Baillie FJ, Morrison AE, Fergus I. Soluble transferrin receptor: a discriminating assay for iron deficiency. Clin Lab Haematol. 2003;25:353–7.
35. Burpee T, Mitchell P, Fishman D, et al. Intestinal ferroportin expression in pediatric Crohn's disease. Inflamm Bowel Dis. 2011;17:524–31.
36. Bonovas S, Fiorino G, Allocca M, et al. Intravenous versus oral iron for the treatment of anemia in inflammatory bowel disease: a systematic review and meta-analysis of randomized controlled trials. Medicine. 2016;95:e2308.
37. Matsumoto T. Platelets in inflammatory bowel disease. J Gastroenterol. 2006;41:91–2.
38. Danese S, Scaldaferri F, Papa A, et al. Platelets: new players in the mucosal scenario of inflammatory bowel disease. Eur Rev Med Pharmacol Sci. 2004;8:193–8.
39. Morowitz DA, Allen LW, Kirsner JB. Thrombocytosis in chronic inflammatory bowel disease. Ann Intern Med. 1968;68:1013–21.
40. Kayo S, Ikura Y, Suekane T, et al. Close association between activated platelets and neutrophils in the active phase of ulcerative colitis in humans. Inflamm Bowel Dis. 2006;12:727–35.
41. Stadnicki A. Involvement of coagulation and hemostasis in inflammatory bowel diseases. Curr Vasc Pharmacol. 2012;10:659–69.
42. Harries AD, Beeching NJ, Rogerson SJ, Nye FJ. The platelet count as a simple measure to distinguish inflammatory bowel disease from infective diarrhoea. J Infect. 1991;22:247–50.
43. Lam A, Borda IT, Inwood MJ, Thomson S. Coagulation studies in ulcerative colitis and Crohn's disease. Gastroenterology. 1975;68:245–51.
44. Talstad I, Rootwelt K, Gjone E. Thrombocytosis in ulcerative colitis and Crohn's disease. Scand J Gastroenterol. 1973;8:135–8.
45. Cabrera-Abreu JC, Davies P, Matek Z, Murphy MS. Performance of blood tests in diagnosis of inflammatory bowel disease in a specialist clinic. Arch Dis Child. 2004;89:69–71.
46. Yuksel O, Helvaci K, Basar O, et al. An overlooked indicator of disease activity in ulcerative colitis: mean platelet volume. Platelets. 2009;20:277–81.
47. Desai D, Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. Aliment Pharmacol Ther. 2007;25:247–55.
48. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut. 2006;55:426–31.
49. Shine B, Berghouse L, Jones JE, Landon J. C-reactive protein as an aid in the differentiation of functional and inflammatory bowel disorders. Clin Chim Acta. 1985;148:105–9.
50. Holtman GA, Lisman-van Leeuwen Y, Reitsma JB, Berger MY. Noninvasive tests for inflammatory bowel disease: a meta-analysis. Pediatrics. 2016;137
51. Sachar DB, Smith H, Chan S, Cohen LB, Lichtiger S, Messer J. Erythrocytic sedimentation rate as a measure of clinical activity in inflammatory bowel disease. J Clin Gastroenterol. 1986;8:647–50.
52. Solem CA, Loftus Jr EV, Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. Inflamm Bowel Dis. 2005;11:707–12.
53. Fagan EA, Dyck RF, Maton PN, et al. Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. Eur J Clin Invest. 1982;12:351–9.
54. Saverymuttu SH, Hodgson HJ, Chadwick VS, Pepys MB. Differing acute phase responses in Crohn's disease and ulcerative colitis. Gut. 1986;27:809–13.
55. Poullis AP, Zar S, Sundaram KK, et al. A new, highly sensitive assay for C-reactive protein can aid the differentiation of inflammatory bowel disorders from constipation- and diarrhoea-predominant functional bowel disorders. Eur J Gastroenterol Hepatol. 2002;14:409–12.
56. Peterson CG, Sangfelt P, Wagner M, Hansson T, Lettesjo H, Carlson M. Fecal levels of leukocyte markers reflect disease activity in patients with ulcerative colitis. Scand J Clin Lab Invest. 2007;67:810–20.
57. Eivindson M, Gronbaek H, Skogstrand K, et al. The insulin-like growth factor (IGF) system and its relation to infliximab treatment in adult patients with Crohn's disease. Scand J Gastroenterol. 2007;42:464–70.
58. Maudgal DP, Ang L, Patel S, Bland JM, Maxwell JD. Nutritional assessment in patients with chronic gastrointestinal symptoms: comparison of functional and organic disorders. Hum Nutr Clin Nutr. 1985;39:203–12.
59. Kane W, Miller K, Sharp HL. Inflammatory bowel disease presenting as liver disease during childhood. J Pediatr. 1980;97:775–8.
60. Gupta N, Cohen SA, Bostrom AG, et al. Risk factors for initial surgery in pediatric patients with Crohn's disease. Gastroenterology. 2006;130:1069–77.
61. Ferrante M, Penninckx F, De Hertogh G, et al. Protein-losing enteropathy in Crohn's disease. Acta Gastroenterol Belg. 2006;69:384–9.
62. Semeao EJ, Jawad AF, Stouffer NO, Zemel BS, Piccoli DA, Stallings VA. Risk factors for low bone mineral density in children and young adults with Crohn's disease. J Pediatr. 1999;135:593–600.
63. Thomas DW, Sinatra FR. Screening laboratory tests for Crohn's disease. West J Med. 1989;150:163–4.
64. Heyman MB, Kirschner BS, Gold BD, et al. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. J Pediatr. 2005;146:35–40.
65. Rubin DT, Mulani P, Chao J, et al. Effect of adalimumab on clinical laboratory parameters in patients with Crohn's disease: results from the CHARM trial. Inflamm Bowel Dis. 2012;18:818–25.
66. Mendes FD, Levy C, Enders FB, Loftus Jr EV, Angulo P, Lindor KD. Abnormal hepatic biochemistries in patients with inflammatory bowel disease. Am J Gastroenterol. 2007;102:344–50.
67. Broome U, Glaumann H, Hellers G, Nilsson B, Sorstad J, Hultcrantz R. Liver disease in ulcerative colitis: an epidemiological and follow up study in the county of Stockholm. Gut. 1994;35:84–9.
68. Feldstein AE, Perrault J, El-Youssif M, Lindor KD, Freese DK, Angulo P. Primary sclerosing cholangitis in children: a long-term follow-up study. Hepatology. 2003;38:210–7.
69. Hyams JS. Extraintestinal manifestations of inflammatory bowel disease in children. J Pediatr Gastroenterol Nutr. 1994;19:7–21.
70. Wilschanski M, Chait P, Wade JA, et al. Primary sclerosing cholangitis in 32 children: clinical, laboratory, and radiographic features, with survival analysis. Hepatology. 1995;22:1415–22.
71. Fumery M, Duricova D, Gower-Rousseau C, Annese V, Peyrin-Biroulet L, Lakatos PL. Review article: the natural history of paediatric-onset ulcerative colitis in population-based studies. Aliment Pharmacol Ther. 2016;43:346–55.
72. Toy E, Balasubramanian S, Selmi C, Li CS, Bowlus CL. The prevalence, incidence and natural history of primary sclerosing cholangitis in an ethnically diverse population. BMC Gastroenterol. 2011;11:83.
73. Sano H, Nakazawa T, Ando T, et al. Clinical characteristics of inflammatory bowel disease associated with primary sclerosing cholangitis. J Hepatobiliary Pancreat Sci. 2011;18:154–61.
74. Cleynen I, Boucher G, Jostins L, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet. 2016;387:156–67.
75. Ridder RM, Kreth HW, Kiss E, Grone HJ, Gordjani N. Membranous nephropathy associated with familial chronic ulcerative colitis in a 12-year-old girl. Pediatr Nephrol. 2005;20:1349–51.
76. Siveke JT, Egert J, Sitter T, et al. 5-ASA therapy and renal function in inflammatory bowel disease. Am J Gastroenterol. 2005;100:501.
77. Van Staa TP, Travis S, Leufkens HG, Logan RF. 5-aminosalicylic acids and the risk of renal disease: a large British epidemiologic study. Gastroenterology. 2004;126:1733–9.

78. Margetts PJ, Churchill DN, Alexopoulou I. Interstitial nephritis in patients with inflammatory bowel disease treated with mesalamine. J Clin Gastroenterol. 2001;32:176–8.
79. De Broe ME, Stolear JC, Nouwen EJ, Elseviers MM. 5-Aminosalicylic acid (5-ASA) and chronic tubulointerstitial nephritis in patients with chronic inflammatory bowel disease: is there a link? Nephrol Dial Transplant. 1997;12:1839–41.
80. Broide E, Dotan I, Weiss B, et al. Idiopathic pancreatitis preceding the diagnosis of inflammatory bowel disease is more frequent in pediatric patients. J Pediatr Gastroenterol Nutr. 2011;52:714–7.
81. Izzedine H, Simon J, Piette AM, et al. Primary chronic interstitial nephritis in Crohn's disease. Gastroenterology. 2002;123: 1436–40.
82. Marcus SB, Brown JB, Melin-Aldana H, Strople JA. Tubulointerstitial nephritis: an extraintestinal manifestation of Crohn disease in children. J Pediatr Gastroenterol Nutr. 2008;46:338–41.
83. Perminow G, Brackmann S, Lyckander LG, et al. A characterization in childhood inflammatory bowel disease, a new population-based inception cohort from South-Eastern Norway, 2005-07, showing increased incidence in Crohn's disease. Scand J Gastroenterol. 2009;44:446–56.
84. Burgmann T, Clara I, Graff L, et al. The Manitoba Inflammatory Bowel Disease Cohort Study: prolonged symptoms before diagnosis–how much is irritable bowel syndrome? Clin Gastroenterol Hepatol. 2006;4:614–20.
85. Jelsness-Jorgensen LP, Bernklev T, Henriksen M, Torp R, Moum BA. Chronic fatigue is more prevalent in patients with inflammatory bowel disease than in healthy controls. Inflamm Bowel Dis. 2011;17:1564–72.
86. Canani RB, de Horatio LT, Terrin G, et al. Combined use of noninvasive tests is useful in the initial diagnostic approach to a child with suspected inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2006;42:9–15.
87. Olives JP, Breton A, Hugot JP, et al. Antineutrophil cytoplasmic antibodies in children with inflammatory bowel disease: prevalence and diagnostic value. J Pediatr Gastroenterol Nutr. 1997;25:142–8.
88. Ruemmele FM, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology. 1998;115:822–9.
89. Dubinsky MC, Johanson JF, Seidman EG, Ofman JJ. Suspected inflammatory bowel disease – the clinical and economic impact of competing diagnostic strategies. Am J Gastroenterol. 2002;97:2333–42.
90. Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. J Pediatr. 1999;134:447–52.
91. Gupta SK, Fitzgerald JF, Croffie JM, Pfefferkorn MD, Molleston JP, Corkins MR. Comparison of serological markers of inflammatory bowel disease with clinical diagnosis in children. Inflamm Bowel Dis. 2004;10:240–4.
92. Zholudev A, Zurakowski D, Young W, Leichtner A, Bousvaros A. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. Am J Gastroenterol. 2004;99:2235–41.
93. Elitsur Y, Lawrence Z, Tolaymat N. The diagnostic accuracy of serologic markers in children with IBD: the West Virginia experience. J Clin Gastroenterol. 2005;39:670–3.
94. Khan K, Schwarzenberg SJ, Sharp H, Greenwood D, Weisdorf-Schindele S. Role of serology and routine laboratory tests in childhood inflammatory bowel disease. Inflamm Bowel Dis. 2002;8:325–9.
95. Benor S, Russell GH, Silver M, Israel EJ, Yuan Q, Winter HS. Shortcomings of the inflammatory bowel disease Serology 7 panel. Pediatrics. 2010;125:1230–6.
96. Reese GE, Constantinides VA, Sim-illis C, et al. Diagnostic precision of anti-*Saccharomyces cerevisiae* antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. Am J Gastroenterol. 2006;101:2410–22.
97. Markowitz J, Kugathasan S, Dubinsky M, et al. Age of diagnosis influences serologic responses in children with Crohn's disease: a possible clue to etiology? Inflamm Bowel Dis. 2009;15:714–9.
98. Young Y, Abreu MT. Advances in the pathogenesis of inflammatory bowel disease. Curr Gastroenterol Rep. 2006;8:470–7.
99. Plevy S, Silverberg MS, Lockton S, et al. Combined serological, genetic, and inflammatory markers differentiate non-IBD, Crohn's disease, and ulcerative colitis patients. Inflamm Bowel Dis. 2013;19:1139–48.
100. Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. Gastroenterology. 2005;128: 2020–8.
101. Papadakis KA, Yang H, Ippoliti A, et al. Anti-flagellin (CBir1) phenotypic and genetic Crohn's disease associations. Inflamm Bowel Dis. 2007;13:524–30.
102. Mow WS, Vasiliauskas EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. Gastroenterology. 2004;126:414–24.
103. 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;101:645–52.
104. Solberg IC, Lygren I, Cvancarova M, et al. Predictive value of serologic markers in a population-based Norwegian cohort with inflammatory bowel disease. Inflamm Bowel Dis. 2009;15:406–14.
105. Zhang Z, Li C, Zhao X, et al. Anti-Saccharomyces cerevisiae antibodies associate with phenotypes and higher risk for surgery in Crohn's disease: a meta-analysis. Dig Dis Sci. 2012;57:2944–54.
106. Dubinsky MC, Lin YC, Dutridge D, et al. Serum immune responses predict rapid disease progression among children with Crohn's disease: immune responses predict disease progression. Am J Gastroenterol. 2006;101:360–7.
107. Dubinsky MC, Kugathasan S, Mei L, et al. Increased immune reactivity predicts aggressive complicating Crohn's disease in children. Clin Gastroenterol Hepatol. 2008;6:1105–11.
108. Lichtenstein GR, Targan SR, Dubinsky MC, et al. Combination of genetic and quantitative serological immune markers are associated with complicated Crohn's disease behavior. Inflamm Bowel Dis. 2011;17:2488–96.
109. Waterman M, Knight J, Dinani A, et al. Predictors of outcome in ulcerative colitis. Inflamm Bowel Dis. 2015;21:2097–105.
110. Dubinsky MC, Mei L, Friedman M, et al. Genome wide association (GWA) predictors of anti-TNFalpha therapeutic responsiveness in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2010;16:1357–66.
111. Arias MT, Vande Casteele N, Vermeire S, et al. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. Clin Gastroenterol Hepatol. 2015;13:531–8.
112. Birimberg-Schwartz L, Wilson DC, Kolho KL, et al. pANCA and ASCA in Children with IBD-Unclassified, Crohn's Colitis, and Ulcerative Colitis-A Longitudinal Report from the IBD Porto Group of ESPGHAN. Inflamm Bowel Dis. 2016;22:1908–14.
113. Joossens S, Reinisch W, Vermeire S, et al. The value of serologic markers in indeterminate colitis: a prospective follow-up study. Gastroenterology. 2002;122:1242–7.
114. Fuchizaki U, Machi T, Kaneko S. Clinical challenges and images in GI. Yersinia enterocolitica mesenteric adenitis and terminal ileitis. Gastroenterology. 2006;131:1379. 659
115. Tuohy AM, O'Gorman M, Byington C, Reid B, Jackson WD. Yersinia enterocolitis mimicking Crohn's disease in a toddler. Pediatrics. 1999;104:e36.
116. Meyer AM, Ramzan NN, Loftus Jr EV, Heigh RI, Leighton JA. The diagnostic yield of stool pathogen studies during relapses of inflammatory bowel disease. J Clin Gastroenterol. 2004;38:772–5.

117. Zilberberg MD, Tillotson GS, McDonald C. Clostridium difficile infections among hospitalized children, United States, 1997-2006. Emerg Infect Dis. 2010;16:604–9.
118. Pascarella F, Martinelli M, Miele E, Del Pezzo M, Roscetto E, Staiano A. Impact of Clostridium difficile infection on pediatric inflammatory bowel disease. J Pediatr. 2009;154:854–8.
119. Martinelli M, Strisciuglio C, Veres G, et al. Clostridium difficile and pediatric inflammatory bowel disease: a prospective, comparative, multicenter, ESPGHAN study. Inflamm Bowel Dis. 2014;20:2219–25.
120. Sands BE. Inflammatory bowel disease: past, present, and future. J Gastroenterol. 2007;42:16–25.
121. Baldassarre ME, Altomare MA, Fanelli M, et al. Does calprotectin represent a regulatory factor in host defense or a drug target in inflammatory disease? Endocr Metab Immune Disord Drug Targets. 2007;7:1–5.
122. 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;34:1357–63.
123. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol. 1992;27:793–8.
124. Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P, Fagerhol MK. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. Lancet. 1990;336:763–5.
125. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scand J Gastroenterol. 1999;34:50–4.
126. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut. 2000;47:506–13.
127. Fagerberg UL, Loof L, Myrdal U, Hansson LO, Finkel Y. Colorectal inflammation is well predicted by fecal calprotectin in children with gastrointestinal symptoms. J Pediatr Gastroenterol Nutr. 2005;40:450–5.
128. Loftus Jr EV. Clinical perspectives in Crohn's disease. Objective measures of disease activity: alternatives to symptom indices. Rev Gastroenterol Disord. 2007;7(Suppl 2):S8–S16.
129. Angriman I, Scarpa M, D'Inca R, et al. Enzymes in feces: useful markers of chronic inflammatory bowel disease. Clin Chim Acta. 2007;381:63–8.
130. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology. 2002;123:450–60.
131. Carroccio A, Iacono G, Cottone M, et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: a prospective study in adults and children. Clin Chem. 2003;49:861–7.
132. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ. 2010;341:c3369.
133. Menees SB, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. Am J Gastroenterol. 2015;110:444–54.
134. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am J Gastroenterol. 2008;103:162–9.
135. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflamm Bowel Dis. 2008;14:32–9.
136. 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;32:171–7.
137. Sidler MA, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. Inflamm Bowel Dis. 2008;14:359–66.
138. Diamanti A, Panetta F, Basso MS, et al. Diagnostic work-up of inflammatory bowel disease in children: the role of calprotectin assay. Inflamm Bowel Dis. 2010;16:1926–30.
139. Degraeuwe PL, Beld MP, Ashorn M, et al. Faecal calprotectin in suspected paediatric inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2015;60:339–46.
140. Henderson P, Anderson NH, Wilson DC. The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol. 2014;109:637–45.
141. Shaoul R, Sladek M, Turner D, et al. Limitations of fecal calprotectin at diagnosis in untreated pediatric Crohn's disease. Inflamm Bowel Dis. 2012;18:1493–7.
142. Henderson P, Casey A, Lawrence SJ, et al. The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory bowel disease. Am J Gastroenterol. 2012;107:941–9.
143. Yang Z, Clark N, Park KT. Effectiveness and cost-effectiveness of measuring fecal calprotectin in diagnosis of inflammatory bowel disease in adults and children. Clin Gastroenterol Hepatol. 2014;12:253–62. e2
144. Canani RB, Terrin G, Rapacciuolo L, et al. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. Dig Liver Dis. 2008;40:547–53.
145. Aomatsu T, Yoden A, Matsumoto K, et al. Fecal calprotectin is a useful marker for disease activity in pediatric patients with inflammatory bowel disease. Dig Dis Sci. 2011;56:2372–7.
146. Zubin G, Peter L. Predicting endoscopic Crohn's disease activity before and after induction therapy in children: a comprehensive assessment of PCDAI, CRP, and fecal calprotectin. Inflamm Bowel Dis. 2015;21:1386–91.
147. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis. 2012;18:2218–24.
148. Walkiewicz D, Werlin SL, Fish D, Scanlon M, Hanaway P, Kugathasan S. Fecal calprotectin is useful in predicting disease relapse in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2008;14:669–73.
149. Gisbert JP, Bermejo F, Perez-Calle JL, et al. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. Inflamm Bowel Dis. 2009;15:1190–8.
150. Sugi K, Saitoh O, Hirata I, Katsu K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. Am J Gastroenterol. 1996;91:927–34.
151. Walker TR, Land ML, Kartashov A, et al. Fecal lactoferrin is a sensitive and specific marker of disease activity in children and young adults with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2007;44:414–22.
152. Pfefferkorn MD, Boone JH, Nguyen JT, Juliar BE, Davis MA, Parker KK. Utility of fecal lactoferrin in identifying Crohn disease activity in children. J Pediatr Gastroenterol Nutr. 2010;51:425–8.
153. Zhou XL, Xu W, Tang XX, et al. Fecal lactoferrin in discriminating inflammatory bowel disease from irritable bowel syndrome: a diagnostic meta-analysis. BMC Gastroenterol. 2014;14:121.
154. Buderus S, Boone J, Lyerly D, Lentze MJ. Fecal lactoferrin: a new parameter to monitor infliximab therapy. Dig Dis Sci. 2004;49:1036–9.