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, 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. Moreover, the blood and stool evaluations may also provide insight into the severity of disease, if indeed IBD (i.e., prognostication), and potentially aid in phenotyping the disease (CD vs. UC). 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" 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 a 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. 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 investigative 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 (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 IBD occurring in both UC [10] and CD [11–17]. Anemia is generally defined as a hemoglobin value <120 g/L or hematocrit <0.4. 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 supplementation [17]. The reported prevalence of anemia is variable in IBD, but anemia appears to be more prevalent in CD compared to UC [18]. In one more recent population-based adult Scandinavian study from Denmark, Norway, and Sweden,

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 [32, 33]
ESR and CRP	Elevation	Nonspecific markers of inflammation, potential role in assessing disease activity, predicting disease relapse and monitoring therapeutic response [42, 57]
Liver function tests	Hypoalbuminemia	*Hypoalbuminemia*: Surrogate marker of nutrition, possibly indicative of decreased liver production (negative acute phase reactant) or intestinal protein losses due to inflammation [21, 57]
	Elevated transaminases Elevated alkaline phosphatase/GGT	*AST/ALT/Alkaline phosphatase/GGT*: Role in evaluating for extraintestinal complications of inflammatory bowel disease [62–64]
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 [98, 99]
Clostridium difficile toxin	Infection	Evaluate for primary infection and co-infection. In patients with inflammatory bowel disease, *C. difficile* is the most common infectious agent identified [10, 100]
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 [110, 111, 114]
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 [110, 111, 114]
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, 89, 92, 93]

ESR erythrocyte sedimentation rate; *CRP* C-reactive protein; *IBD* inflammatory bowel disease; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *GGT* Gamma glutamyl transpeptidase; *ASCA* anti-*S*accharomyces cerevisisae (*ASCA*); *pANCA* perinuclear anti-nuclear cytoplasmic antibody; *OmpC* outer membrane protein

the overall prevalence of anemia was 19% with iron deficiency and anemia of chronic disease being the primary etiologies [18]. Additionally, anemia may be more common in children compared to adolescents and adults [19]. Using the WHO age-adjusted definitions of anemia, Goodhand et al., [19] 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 recent Saudi Arabian study, anemia was found in 86% of children affected by either UC or CD [20]. 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, 19, 21–24] and 9–67% of patients with UC (30% reported in one pediatric cohort) [17, 21, 24].

The cause of iron deficiency with or without frank anemia is likely multifactorial in both CD and UC [25]. 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 [25]. 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 [25]. The anemia observed in UC 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. 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 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, 26]. 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 [27]. In this particular study [27], 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 that 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. 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 [28–30].

Soluble transferrin receptor concentration, which is not affected by inflammation, is elevated in iron deficiency anemia, but remains normal in anemia of chronic disease [28–30]. In addition to soluble transferring receptor, intestinal ferroportin expression should be considered as a marker of anemia in relationship to IBD and, particularly, CD in children. In a recent study performed by Burpee et al., [31] 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 ($n=19$) and ten were without CD. The authors observed that intestinal ferroportin protein was higher in anemic CD subjects than in non-anemic CD subjects, while 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].

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 (e.g., juvenile rheumatoid arthritis) [32, 33]. 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., [34] 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. However, more recently, 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. [35] 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 [35]. There is also evidence

that coagulation activation may mediate and amplify inflammatory cascades in IBD, especially via activating proteinase-activated receptor-related pathways [36]. Patients with CD and UC are at least 3–4-fold increased risk of developing thromboembolic (TE) complications compared to control patients [36]. Although the etiology is multifactorial, TE phenomena in IBD is largely attributable to coagulation activation and platelet aggregation during systemic inflammation [36]. 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. TE processes) of the system inflammation of IBD, rather than being a simple "biomarker" of IBD [32, 36].

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 [21]. 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 [37–39]. 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 [40]. 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 [37].

Mean platelet volume (MPV) is influenced by the degree and type of mucosal and system inflammation. A recent study analyzed overall accuracy of MPV in disease activity and compared MPV with other inflammatory markers in 61 UC patients and 27 healthy subjects [41]. 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 in active UC patients than controls [41]. 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 and c-Reactive Protein and Other Markers

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are two other nonspecific measures of inflammation which should be included in the evaluation of patients with

suspected IBD [42]. 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 correlate well with disease activity, and thus is one objective marker that may be helpful in distinguishing IBD from noninflammatory conditions [43]. 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 [43]. 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, 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 [21]. 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 [44]. Therefore using these markers in combination may increase the diagnostic yield.

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 [45–48]. However, the development of highly sensitive CRP assays may improve the sensitivity of this test, even in patients with limited disease [49]. In a study by Poullis et al. [49] 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 cut-off 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 [49]. 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 therefore may be a better measure of remission and response to therapy than other inflammatory markers in patients with IBD [43].

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 [43]. 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 [50]. Blood samples were analyzed for MPO, EPX, CRP, 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 of the 28 patients [50]. Levels of fecal markers correlated with endoscopic scores, histological severity, and circulating blood acute-phase reactants, i.e., orosomucoid [50]. In one small study of Scandinavian adults with CD undergoing infliximab therapy, Crohn disease Activity Index, the Harvey Bradshaw Index, CRP, as well as orosomucoid and albumin reached normal levels during infliximab treatment [51]. Orosomucoid was as sensitive as the more "traditional" inflammatory markers and correlated tightly with physician global assessment and CDAI [51].

More recently, a study demonstrated a novel marker of intestinal inflammation, namely neutrophil gelatinase-associated lipocalin (NGAL), which may be an equivalent if not better marker for disease than ESR or CRP [52]. NGAL is a multi-potent 25-kDa protein mainly secreted by neutrophils and NGAL over-expression in the colonic epithelium has been described in patients with IBD. In this study, NGAL obtained from serum samples displayed a strong ability to distinguish active IBD from inactive disease [52]. NGAL was also able to clearly differentiate IBD (active or inactive) patients from healthy controls, or IBS patients, performing better than ESR and CRP in the assessment of disease activity in both UC and CD [52]. Although promising, more studies are clearly needed with NGAL comparing active IBD from inactive disease and using it to differentiate IBD from non-IBD, particularly in pediatric populations wherein this marker could be hypothesized to be more sensitive.

In a similar fashion, a group of investigators recently took a novel approach and looked at plasma amino acid profiles in order to distinguish patients with IBD from non-IBD, and, to distinguish CD from UC [53]. The investigators measured fasting plasma aminograms in a total of 387 IBD patients (CD, $n=165$; UC, $n=222$) and 210 healthy controls. The authors employed their biostatistical model, multivariate indexes established from plasma aminograms, and were able to distinguish CD or UC patients from healthy controls and were also able to distinguish active CD or UC patients from remission patients. Moreover, the plasma aminograms correlated tightly with traditional clinical disease activity indexes for CD or UC [53]. 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 and in the determination of the IBD phenotype—i.e., presence or absence of extra-intestinal manifestations such as liver disease [54, 55]. 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 [55]. 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 [21, 22, 56–60]. In a more recent, 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 [20]. 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 [56] as well as for osteopenia and decreased bone mineral density scores [58]. Albumin can also be used as a marker for response to therapy. In an adult multicenter clinical trial evaluating one of the newer biologics for therapy of CD, the authors investigated the effect of adalimumab on changes in laboratory values using data from CHARM trial [61]. 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 [61].

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 [46, 57, 59].

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., [62] 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 [62]. 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 [63].

However, when AST/ALT are persistently elevated or seen in association with an elevated ALP, elevated direct bilirubin and/or γ-glutamyl transpeptidase, the extraintestinal complication of primary sclerosing cholangitis (PSC) or autoimmune hepatitis/overlap syndrome should be considered. PSC is a reported complication in 3% of children with IBD and can precede or occur coincident with diagnosis of IBD [64–66]. In a recent US 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 [67]. 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, $p=0.005$) [67]. In another small-sized single-center study ($N=29$), the incidence of IBD in PSC patients was 68.9% (20/29) [68]. 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, $p<0.001$) [68]. With regards to pediatric patients, Wilschanski et al [66]. demonstrated of 32 children with PSC, that the majority of patients 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 IBD, all with colitis, 14 UC, and 3 CD [66]. 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., [64] 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 [64]. 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 US 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 [64].

Renal as well as pancreatic disease may also be important extra-intestinal manifestations of IBD or can be adverse events associated with IBD pharmacotherapy [69–74]. 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). These authors demonstrated that 10 of 460 pediatric patients with IBD (2.17%), compared with only 2 in 3,500 adults (0.06%) presented with pancreatitis. Eight children had colonic disease (4 CD, 4 UC (3 pancolitis)) with the mean amylase level being 1,419 (range 100–1,370) and three children (30%) having mildly elevated transaminases. Of note was that median time between onset of first episode of acute pancreatitis in relation to onset of IBD was 24 (range 1–156) weeks and the most common presentation was abdominal pain. Similarly, renal disease may precede diagnosis of IBD. Although small in sample size, Izzedine et al. [75] described 4 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 3 of the 4 patients [75]. A similar small case series of 2 pediatric patients with renal disease occurring concurrently with diagnosis of IBD has been reported [76]. 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 CD 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 obtained 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-mentioned details 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 UC and CD from functional bowel disorders and infectious etiologies of their presenting signs and symptoms. 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 CD 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 [77–79].

Several other noninvasive studies have been proposed to aid in the diagnosis of IBD 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 cerevisisae* (ASCA), an antibody response against *Saccharomyces cerevisiae* and perinuclear anti-nuclear 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, 80–89]. 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 [82, 84–87] and pANCA specificity ranged from 65 to 95% for UC [81, 82, 84–87]. 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, 82, 84, 88]. Unfortunately, the sensitivity of these serologies has been shown to be poor with overall sensitivity ranges reported between 55 and 78% [1, 5, 80, 82, 84, 88]. 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 CD as 55 and 93%, respectively [90]. The sensitivity and specificity of positive pANCA for detection of UC were lower at 55.3 and 92.8%, respectively [90], 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 Omp-C, an antibody to the outer membrane porin of *Escherichia coli*, appears to add little to the diagnostic accuracy of this serologic panel in children [86, 87]. In two pediatric studies, the overall sensitivity of anti-OmpC for both CD and UC was very low [86, 87]. However, the use of the additional IBD serologies may help identify a small number of IBD patients who had negative ASCA and pANCA [86, 87]. 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 [91]. 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 [91].

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. [88] 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 vs. 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 CBir1 flagellin to the serological panel does not appear to improve the diagnostic yield of this panel. A recent 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 [89]. 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% [89]. 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., [92] 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. [93] also demonstrated that anti-CBir1 serum reactivity in CD patients is independently associated with fibrostenosing disease and complicated small bowel CD. Anti-Omp C and anti-IL2 have also been shown to be associated with more aggressive disease course in adults patients with CD [94]. 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 [95, 96]. Similar to adult data, anti-Omp C and anti-IL2 were independently associated with these complications [95]. 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 [97]. Based on the above data, perhaps for now, the use of these serologies should be reserved as an aid in classifying disease subtype in children with indeterminate colitis, assisting in therapeutic decisions such as colectomy, and potentially as a prognosticator of disease course.

Stool Evaluation

The presentation of pediatric IBD 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 of the child to differentiate infectious vs. 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 [98, 99]. Also, defects in mucosal barrier function can predispose patients with IBD to infectious colitis, and *Clostridium difficile* is the most common infectious agent identified [10, 100].

In addition to differentiating between infectious colitis and IBD, there has been a lot of recent attention toward 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, 101]. Moreover, there has been a resurgence of interest in the organism *Mycobacterium avium* subspecies *paratuberculosis* and its role as a trigger, and/or modulator of IBD, particularly CD [102–104]. This renewed interest has been specifically fueled by a recent report of a several-month course of triple therapy antibiotics which successfully resolved CD at short-term follow-up in a small cohort of Australian adults [103]. 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.

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 [105–107].

Calprotectin has bacterostatic and anti-fungal properties, and thus likely contributes to neutrophilic defenses [108]. In healthy individuals, concentrations of calprotectin are approximately six times higher in stool than plasma [107]. 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 [109, 110]. 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 [42, 107, 111–113].

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 [42, 111–113]. 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 [114]. This test was more sensitive than either ESR or CRP and an abnormal fecal calprotectin had an odds ratio for disease of 27.8 [114]. A similar, but small cohort-sized study by Carroccio et al. [115] 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 IBD, the sensitivity and specificity of fecal calprotectin was 100 and 95%, respectively [115]. A subsequent meta-analysis of 6 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 [116]. 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 [117, 118].

There have also been several studies evaluating fecal calprotectin in the pediatric population. Carroccio et al.'s [115] 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. [111] 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. [119] 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 IBD. 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 [120, 121], and a meta-analysis of seven prospective pediatric studies ($n = 371$) revealed a pooled sensitivity and pooled specificity of 92 and 76%, respectively [116]. Based on these collective results, it appears that fecal calprotectin correlates well with the presence of histologic inflammation in pediatric patients.

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 [122, 123]. Additionally, there have been several pediatric 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 had clinical relapse whereas 89% with fecal calprotectin below this threshold remained in clinical remission [124]. A larger prospective pediatric study also demonstrated that calprotectin concentrations in patients who relapsed were higher than those who did not, with a fecal calprotecin level of >150 μg/g having a sensitivity of 69% and specificity of 69% to predict relapse [125]. Therefore, the assay offers an advantage over other nonspecific inflammatory markers as it 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.

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 [117, 118]. 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 [126]. 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 [127]. Pfefferkorn et al. [128] 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 [128]. Thus fecal lactoferrin appears to be another promising noninvasive marker of intestinal inflammation, and therefore a consideration in evaluation of patients with suspected IBD. Lactoferrin may also be useful to monitor response to therapy, and in one small case series of five pediatric patients with CD, fecal lactoferrin significantly decreased in response to infliximab therapy [129].

Summary

In the preceding sections, we attempted to provide an overview of the laboratory tests available that can be used in the initial work-up of the child with suspected IBD. 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, 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 vs. 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 with the child with IBD, which ultimately helps guide the clinician toward more invasive testing.

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