The utility of fecal lactoferrin measurements in predicting disease activity of hospitalized patients with ulcerative colitis

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THE UTILITY OF FECAL LACTOFERRIN MEASUREMENTS IN PREDICTING DISEASE ACTIVITY OF HOSPITALIZED PATIENTS WITH ULCERATIVE COLITIS

by

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DEDICATION

This project is dedicated to the memory and life of Peter Harris, whose immense passion and dedication to every aspect of his life inspires me every day of my life.
ACKNOWLEDGEMENTS

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KELLEN F. MANDEHR

ABSTRACT

Background: Early identification of pediatric patients with Inflammatory Bowel Disease (IBD), including ulcerative colitis and Crohn disease, is important to help clinicians design optimal treatment regimens. Existing endoscopic techniques are effective in identifying disease activity. However, these methods are invasive, expensive, and less amenable to serial measurement. Recent studies have identified potential serologic and fecal biomarkers that may have the potential to provide clinicians with a more objective evaluation of disease activity. In the case of ulcerative colitis (UC), in which disease is confined to the large intestine, the information provided by fecal biomarkers is likely to be more specific than that provided by serologic biomarkers. Fecal lactoferrin (FLA) is one such biomarker that has shown to be useful not only in identifying levels of colonic inflammation, but also for use as a predictor of disease relapse and treatment efficacy. Measurement of fecal lactoferrin, in conjunction with information provided by other diagnostic modalities could expedite patient assessment and treatment. Additionally, it has been suggested that fecal lactoferrin levels may also provide prognostic information about response to treatment and disease outcome in pediatric patients with UC. The goal
of this study is to explore the relationship between changes in FLA levels and response to medical therapy in hospitalized pediatric patients with UC.

Methods: Serial stool samples were collected daily from 10 patients admitted for management of severe active UC. Of these 10 patients, 3 responded favorably to standard treatment with intravenous corticosteroid therapy and were discharged to complete a course of oral steroids. 7 were unresponsive to steroid therapy and went on to require rescue (more intensive) medical therapy. Changes in FLA were correlated with steroid response and medical disposition at the time of discharge.

Results: A t-test was performed to determine the significance of the differences in percent change in FLA levels between patients discharged on steroids and patients discharged on rescue therapy. Patients discharged on steroids demonstrated a net decrease in FLA levels over the course of the first three days of steroid treatment while patients ultimately requiring rescue medical therapy demonstrated a net increase in FLA levels (mean values = -64.4% and +203.8%, respectively). A difference was found between the averages; however, this value did not reach statistical significance when analyzed with a t-test (p = 0.18).
Conclusions: This study suggests that quantitative FLA levels may prove useful in predicting clinical course and discharge outcome in pediatric patients with ulcerative colitis. Future research in this field should seek larger sample sizes, increased longitudinal sample collection, and the potential for a composite assessment that will yield additional objective measures of disease activity.
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<th>Description</th>
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<tr>
<td>5-ASA</td>
<td>5-Aminosalicylate</td>
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<tr>
<td>6MP</td>
<td>6-mercaptopurine</td>
</tr>
<tr>
<td>ASCA</td>
<td>Anti-saccharomyces Cerevisiae Antibody</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen-Presenting Cell</td>
</tr>
<tr>
<td>AZA</td>
<td>Azathioprine</td>
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<tr>
<td>CD</td>
<td>Crohn Disease</td>
</tr>
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<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
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<tr>
<td>FC</td>
<td>Fecal Calprotectin</td>
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<td>FLA</td>
<td>Fecal Lactoferrin</td>
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<tr>
<td>GALT</td>
<td>Gut-Associated Lymphoid Tissue</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable Bowel Syndrome</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>MALT</td>
<td>Mucosa-Associated Lymphoid Tissue</td>
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<tr>
<td>NSAID</td>
<td>Non-steroidal Anti-inflammatory drug</td>
</tr>
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<td>NF-kB</td>
<td>Nuclear Factor kappa-light-chain-enhancer of activated B cells</td>
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<td>Abbreviation</td>
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<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
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<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-alpha</td>
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<td>UC</td>
<td>Ulcerative Colitis</td>
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INTRODUCTION

Inflammatory Bowel Disease (IBD) refers to a collection of idiopathic chronic inflammatory conditions affecting the gastrointestinal tract. IBD arises most often during late childhood and early adulthood, demonstrating a peak incidence between the ages of 15 and 30 years. Approximately 25% of patients are diagnosed during childhood (Benchimol, et al. 2011). Two major subtypes of IBD exist, including Crohn disease (CD) and ulcerative colitis (UC) (Medline, 2012). The pathophysiology of both disorders is characterized by chronic inflammation as the cardinal feature. However, they can be distinguished based on clinical features, disease distribution, and endoscopic/histologic appearance. More specifically, UC is limited to the mucosal layer of the large intestine. In contrast, inflammation in patients with CD often penetrates to involve the submucosa or serosa of the bowel wall and can affect virtually any part of the gastrointestinal tract, from the mouth to the anus (Higuchi & Bousvaros, 2013). The pathogenesis of neither disorder has been fully elucidated. However, data from recent studies suggests that the inflammatory response observed in patients with UC and CD may result from a combination of genetic vulnerability in the context of an inappropriate activation of the intestinal immune response against bacteria native to the gut (Saeed & Kugathasan, 2008). A second hypothesis regarding the development of IBD relates to what is now referred to as the “Hygiene Hypothesis”. In this current “hyper-hygienic” age, children are now born and raised in the context of decreased and/or restricted microbial challenges. As a result, our highly evolved, yet now underused, immune system is more
likely to respond aberrantly to native (self) antigens. This may explain the recent increased incidence in autoimmune diseases including IBD, asthma, food allergies, and arthritis (Saeed and Kugathasan, 2008). While many hypotheses exist regarding IBD causation, many more questions regarding its pathogenesis remain unanswered, making evaluation and treatment of these patients difficult.

**Clinical Features of the IBD**

Patients with UC and CD can present with similar clinical pictures. As such, clinicians typically rely on differences in the anatomic location or the nature of the mucosal inflammation to characterize a patient’s clinical signs and symptoms as being most consistent with either UC or CD. UC is characterized by diffuse inflammation, beginning in the rectum and extending up in a confluent fashion to include part or all of the large intestine. In contrast, inflammation in patients with CD is more often seen as discontinuous, and manifests “skip lesions” (Abraham & Cho, 2009; Hendrickson, et al. 2002). Macroscopically, the inflammation in UC is typically restricted to the superficial mucosa, while the inflammation in patients with CD can be penetrating (i.e. extending through the mucosa to include the submucosa and serosa of the intestine) (Higuchi & Bousvaros, 2013). Chronic and penetrating inflammation of this type can result in stricturing (i.e. narrowing of the intestine), perforation, or the development of fistulas (a pathologic connection of one portion of the intestine to another) (Hendrickson et al., 2002). The macroscopic features used to help differentiate patients with UC from those with CD are presented in Table 1 (Glickman, 2005).
The most common presenting clinical symptoms in patients with IBD include abdominal pain, diarrhea, and decreased appetite, (Mamula et al., 2003). One dimension that can be used to help distinguish between UC and CD is the gross appearance of the patient’s stool. The presence of blood and mucus in the stool is more likely to point to a diagnosis of UC (Bousvaros et al., 2007). UC patients might also present with acute systemic symptoms including tachycardia, fever, or dehydration. In contrast, the penetrating nature of the inflammation in patients with CD can result in patients presenting with perianal abscesses (Higuchi & Bousvaros, 2013) or intestinal stricturing. Small bowel inflammation can also result in intestinal dysfunction and decreased nutrient absorption. Over the long-term, this malabsorption can result in patients with CD presenting with more indolent systemic signs and symptoms including poor weight gain, decreased linear growth, and delayed pubertal development (Mamula et al., 2003). As such, the effects of IBD can have a greater impact on younger children, who are especially vulnerable to any alterations in growth patterns (Kelsen & Baldassano, 2008).

Table 1: Pathological Features Useful in Distinguishing Ulcerative Colitis and Crohn Disease. This table displays some of the common clinical findings physicians use in differentiating patients with UC from those with CD. Taken and adapted from Glickman, 2005.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Ulcerative Colitis</th>
<th>Crohn Disease</th>
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<tr>
<td>Inflammation</td>
<td>Continuous</td>
<td>Discontinuous</td>
</tr>
<tr>
<td>Rectal involvement</td>
<td>Always</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Ileal involvement</td>
<td>Not typical</td>
<td>Frequent</td>
</tr>
<tr>
<td>Upper gastrointestinal tract involvement</td>
<td>Sometimes</td>
<td>Frequent</td>
</tr>
<tr>
<td>Fissures</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Transmural involvement</td>
<td>Absent</td>
<td>Frequent</td>
</tr>
<tr>
<td>Tissue granulomas</td>
<td>Absent</td>
<td>Present</td>
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</table>
Epidemiology of Inflammatory Bowel Disease

IBD has historically been most prevalent in Western societies including the US, UK, and Scandinavia. However recent epidemiologic studies have demonstrated an increased prevalence on a global scale. To this end, patients with IBD are now more frequently identified in Asia, Latin America, Africa, and part of Southern Europe (Loftus, 2004; Griffiths, 2004; Benchimol et al., 2011). The increasing incidence of IBD in less developed countries is believed to be caused by cultural “Westernization”, including fundamental changes in dietary and other lifestyle habits (Loftus, 2004). Other demographic factors relevant to IBD include gender. Women appear to have a higher incidence of CD than men, while the opposite has been reported in patients with UC (Benchimol et al., 2011). With respect to racial and ethnic factors, there has been a significant increase in IBD diagnoses among African-American patients. In contrast, Asian, Latino, and Native American populations continue to demonstrate inexplicably lower incidence rates (Loftus, 2004). There appears to be a significant age or developmental component to the pathogenesis of IBD as well. While the ratio of CD to UC patients in adult populations is close to 1:1, the ratio in children is much closer to 3:1 (Sauer & Kugathasan, 2009).
**Etiology of Inflammatory Bowel Disease**

The pathogenesis of IBD appears to be the end result of a combination of genetic and environmental factors. Currently, twin studies provide the most readily interpretable genetic evidence as stated by Benchimol et al., 2011:

Twin studies have shown that inherited genetic risk factors alone play a small role in the pathogenesis of IBD (16%-36% concordance rates in monozygotic twins and 4% concordance rates in dizygotic twins).

The concordance rate appears to be higher in patients with CD than in those with UC. Overall, the risk of developing IBD is believed to be up to 14-15 times greater in first-degree relatives (Binder, 1998).

Environmental risk factors are also relevant in the pathogenesis of IBD (Saeed & Kugathasan, 2008). Some of the most significant and best-studied environmental factors associated with the development of IBD include smoking, appendectomy, diet, and environmental disturbances of the internal microbiome (Saeed & Kugathasan, 2008; Loftus, 2004; Ng et al., 2013). Interestingly, the effect of smoking on the clinical course of IBD appears disease-specific. Smoking has been shown to have an inverse relationship with the development of UC, while smoking appears to increase disease severity in patients with CD. Likewise, appendectomy is seen as a protective measure from developing UC, while some studies have shown a correlation between appendectomy and increased CD (Loftus, 2004).

The role of the internal intestinal flora in the development of IBD is yet to be fully elucidated, but the issues are outlined by Ng et al. (2013) and Frank et al. (2007) in their papers:
The dysbiosis reported in IBD is likely to be a consequence of changes in environment including hygiene and food, resulting in imbalance in the microbial-host relationship with mucosal barrier dysfunction and reduced microbial diversity. Indeed, diet is seen as an important modifier of an individual’s gut flora. In keeping with the “hygiene hypothesis”, it is thought that children born in "westernized" areas of low microbial exposure are more prone to developing IBD than those in high-exposure regions because appropriate tolerance is not established early in life (Saeed & Kugathasan, 2008). Two other dietary concepts are equally noteworthy. First, the advent of refrigeration (referred to as the “cold-chain hypothesis”) has led to the prolonged life span of food and consequently, the bacteria that colonizes it (Loftus, 2004). Second, the revolution in antibiotic development and widespread use may be leading to increased incidence of IBD through alterations in gut flora (Ng et al., 2013). As changes in diet, antibiotic exposure, and food preparation/storage have all mirrored the increase in IBD, additional studies are going to be necessary to discern the complex interplay between genetic and environmental factors (Mamula et al., 2003).

Pathogenesis of Inflammatory Bowel Disease

Mucosa-associated lymphoid tissue (MALT) comprises a significant portion of the human immune system and resides in mucosal surfaces lining the body. More specifically, this includes the gut-associated lymphoid tissue (GALT), which is found in the intestinal mucosa and is responsible for protecting the body from infective and atopic enteric challenges (Medscape, 2012). GALT is the largest collection of lymphoid tissues in the body and consists of both organized lymphoid tissues, including mesenteric lymph
nodes and Peyer’s patches, as well as IgA-producing lymphocytes that are distributed throughout the intestinal lamina propria (Forchielli & Walker, 2005). Dendritic cells, which are highly specialized antigen-presenting cells, also reside within the intestinal mucosa and participate in immune surveillance and help to orchestrate immune responses by activating specific T-lymphocyte populations (Tezuka & Ohteki, 2010).

The sequence of immune events in mediating or propagating the inflammation observed in patients with IBD is not certain. However, it is clear that an interaction between the GALT, the intestinal epithelium, and the commensal intestinal flora is crucial (Abraham & Cho, 2009). Poorly regulated mucosal immune responses, likely resulting from aberrant bacterial signaling from the intestinal lumen, are a potential risk factor for ongoing bowel inflammation (Forchielli & Walker, 2005). Commensal bacteria can act to limit proinflammatory signaling pathways that are mediated by cell-surface Toll-like receptors (TLR) and cytosolic NFkB proteins. Without these interactions, immune tolerance of gut flora cannot develop appropriately, and this can help to drive intestinal inflammation (Tezuka & Ohteki, 2010). In patients with IBD, this inflammation is chronic and leads to progressive epithelial injury and ulcerations (Abraham & Cho, 2009).

**Diagnostic Techniques and the Importance of Disease Differentiation**

Patients with CD and UC can present with many of the same clinical features. However, differences in the pathogenesis, as well as the nature of the inflammatory responses observed in these disorders, can lead to differing complications and prognoses
As such, the frequently indolent nature of the inflammation observed in patients with CD leaves them more vulnerable (60-88% of patients) to growth failure and delays in sexual maturation than in patients with UC (6-12% of patients) (Hendrickson et al., 2002). Therefore, there is a clear premium on identifying disease at an early age or early in the disease course to prevent these potentially irreversible clinical complications (Sauer & Kugathasan, 2009).

Endoscopic study with mucosal biopsy and radiological study are accepted as the standard of care in evaluating children with suspected IBD. Endoscopy with biopsy can be definitive in differentiating patients with CD from those with UC, given differences in the endoscopic and histologic appearance of involved mucosa (Hendrickson et al., 2002; Bousvaros et al., 2007; Higuchi & Bousvaros, 2013). Radiologic studies permit an assessment of small bowel loops that are typically inaccessible using standard endoscopic/colonoscopic techniques (Hendrickson et al., 2002). While these studies are considered the “gold standard” in diagnostic testing, their sensitivity and specificity parameters fall short of 100% (Sherwood, 2012). As a result, studies are underway to define more expedient and accurate ways of diagnosing and differentiating patients with IBD (Bousvaros et al., 2007).

Treatment options

The range of options available to clinicians to treat their patients with IBD has greatly increased. Nonetheless, there is little in the way of evidence-based data to formulate a unified/standard clinical approach to the management of patients with either
CD or UC (Sauer & Kugathasan, 2009). Medications used to treat patients with IBD generally fall into three categories: those used to induce disease remission, those used to maintain disease remission, and those used in patients with refractory disease (so called “rescue therapy”). Surgery is typically reserved for those patients that have failed medical therapy or developed a complication, including perforation, obstruction, and growth delay (Sauer & Kugathasan, 2009). Induction agents are more potent and typically used short term due to the potential for detrimental long-term side effects. Corticosteroids (administered either orally or intravenously) are the most widely used class of medications for inducing remission in patients with either UC or CD. 5-aminosalicylates (ASAs) have shown to be effective in inducing remission in patients with mild-moderate UC (Higuchi & Bousvaros, 2013). Clinicians often use immunomodulators, including 6-mercaptopurine (6MP) or Azathioprine (AZA), to maintain CD and UC disease remission. In instances in which patients fail to respond to steroid and/or immunomodulation therapy, clinicians may choose to employ rescue therapy with more potent immunosuppressive drugs like tacrolimus (Sauer & Kugathasan, 2009; Higuchi & Bousvaros, 2013). The recent advent of biologic immunomodulation using antibodies to TNF-α has been proven effective for treating patients with CD and UC (Abraham & Cho, 2009). In the event of complete medical treatment failure, surgery is typically advised. In patients with UC, total colectomy is recommended. In contrast, patients with refractory CD are typically treated with isolated small or large bowel segmental resection (Higuchi & Bousvaros, 2013).
Treatment Course

The course of treatment for pediatric patients presenting with moderate to severe UC can vary from center to center (Sauer & Kugathasan, 2009). Within the scope of this variability, IBD Centers including Boston Children’s Hospital follow a commonly used treatment plan for patients that exhibit disease flares. Typically, children admitted with moderate to severe colitis will initially be treated with intravenous corticosteroids, possibly with adjunct therapy (antibiotics). Patients responding to this course of therapy (approximately 80%) will be discharged to complete a course of oral steroid therapy and a maintenance medication (ASA, immunomodulator, or biologic). If it becomes apparent after 5-10 days that the patient is not responding to steroid therapy, it becomes necessary to discuss more aggressive treatment, including the use of more potent (rescue) immunosuppressive therapies or surgery (colectomy) (Higuchi & Bousvaros, 2013). Rescue medications currently include tacrolimus, infliximab, and cyclosporine. Previous data suggest that 80% of patients failing to respond to corticosteroid therapy will ultimately respond to rescue therapy. Those failing rescue therapy are typically referred for surgery. Patients responding to rescue therapy are transitioned to maintenance agents including immunomodulators or infliximab for maintenance (Higuchi & Bousvaros, 2013; Sauer & Kugathasan, 2009). As such, there is a pressing need to develop newer generations of biomarkers and treatment algorithms to assist clinicians in discerning which patients are likely to respond to steroid therapy and which are likely to go on to rescue or surgery. Earlier identification of these refractory patients will shorten
hospitalization; decrease morbidity related to central venous access and parenteral nutrition; and lower overall healthcare costs.

**Serological and Fecal Biomarkers**

There are many diagnostic modalities currently available for use in evaluating patients with suspected IBD. However, many of these technologies are limited by cost, inconvenience, or patient morbidity. Hence, clinicians continue to seek novel methods that are less invasive, more specific, and less costly for use in the diagnosis of patients with IBD (Kane, Sandborn, Rufo et al., 2003; Assche, 2011). To that end, efforts are currently underway to develop and validate specific serologic or fecal biomarkers that can be used to reduce dependence on more costly and invasive studies, assess disease activity and disease severity more directly, and assist clinicians in predicting the likelihood for disease relapse in individual patients (Masoodi et al., 2011). Biomarkers may also provide more valid objective data when used in conjunction with existing subjective clinical disease activity indices (Assche, 2011).

The most well known serological markers that have been employed by clinicians to assess disease activity in patients with IBD include C-reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) (Masoodi et al., 2011). However, both measures lack disease specificity and can be influenced by infection or inflammation in any part of the body. As such, fluctuations in these values are less useful in IBD patients with concomitant illnesses (Assche, 2011; Abraham & Kane, 2012; Sherwood, 2012). Nonetheless, until more specific biomarkers for IBD have been identified and validated,
clinicians will continue to rely on ESR and CRP to help assess for active inflammation in patients with UC and CD (Assche, 2011; Sherwood, 2012).

Another serological marker that has been in widespread use for assessing patients with suspected IBD is Anti-\textit{Saccharomyces cerevesiae} antibody (ASCA). Existing data suggest that this serologic antibody to a commensal yeast may be a useful tool in identifying patients with IBD and distinguishing patients with CD from those with UC. While ASCA has demonstrated higher specificity and sensitivity than other serological antibodies, data from this assay is not sufficiently reliable to be used for diagnostic purposes and subsequent endoscopic studies are still mandated. As such, efforts are underway to define the clinical contexts in which serologic ASCA measurements may prove most useful (Reese et al., 2006; Prideaux, et al., 2011, Gisbert et al., 2009).

The application of fecal biomarkers (lactoferrin and calprotectin) for use in the diagnosis and interval assessment of patients with IBD is becoming increasingly widespread. This technology has the advantages of being generally inexpensive, non-invasive, and amenable to serial measurement in the same patient over time (Masoodi et al., 2011). Unlike serum biomarkers, fecal biomarkers are a product of the mucosal immune system of the GI tract, and are therefore much more sensitive and specific than their serologic counterparts in assessing inflammation of the intestinal mucosa in patients with IBD (Assche, 2011; Masoodi et al., 2011). Additionally, fecal biomarkers are likely to be more accurate than serological testing in differentiating patients with an inflammatory process (IBD) from those with non-inflammatory GI conditions like Irritable Bowel Syndrome (IBS) (Abraham & Kane, 2012; Masoodi et al., 2011).
However, while elevated fecal lactoferrin and calprotectin levels are sensitive to mucosal inflammation, they are generally unable to discern if the intestinal inflammation being evaluated is the result of an autoimmune (e.g. IBD) or an acute infectious (e.g. Salmonella, Shigella, Yersinia, Campylobacter, and/or E. coli) process. Additionally, existing fecal biomarkers do not differentiate between CD and UC (Abraham & Kane, 2012).

**Fecal Calprotectin**

One biomarker in widespread use for evaluation of intestinal inflammation is fecal calprotectin (FC), a calcium-binding protein with antibacterial, antifungal, and metalloproteinase-inhibitory properties (Masoodi et al., 2011; Beniwal & Harrell, 2010). FC is derived from neutrophil granules, so the degree of inflammation present in the intestine is believed to correlate directly with the levels of FC (Abraham & Kane, 2012; Masoodi et al., 2011; Sherwood, 2012). Recent findings further indicate that FC correlates with endoscopic disease activity better than preexisting activity indices and serum biomarkers (Schoepfer et al, 2013). While FC is viewed as a reliable indicator of inflammation, a number of factors must be considered in interpreting any results. Specifically, FC levels may be affected by NSAID use and blood loss exceeding 100mL of total blood volume (Masoodi et al., 2011; Abraham & Kane, 2012). Due to these potential confounding variables, clinicians are looking towards other fecal biomarkers to reflect not only disease activity but also provide better indication of treatment response or the need for escalating clinical intervention.
**Fecal Lactoferrin**

Fecal Lactoferrin (FLA) is a well-established fecal biomarker of inflammation. FLA is a small iron-binding glycoprotein that, like calprotectin, is also found in neutrophil granules and body fluids including breast milk, tears, and saliva. FLA displays bactericidal properties in-vitro and in-vivo and has evolved to play an intrinsic role in innate immunity (Kane et al., 2003; Gisbert et al., 2009). Previous data has demonstrated that changes in the level of this fecal biomarker can be useful in assessing the degree of inflammation in patients with IBD (Masoodi et al., 2011; Walker et al., 2007). To that end, FLA levels are significantly higher in patients with IBD than those with Irritable Bowel Syndrome (Basso et al., 2013, Abraham & Kane, 2012; Beniwal & Harrell, 2010). Ongoing studies should assist in discerning whether FLA might also be useful in monitoring disease activity, assessing therapeutic efficacy, and predicting disease relapse in patients with IBD.

Ultimately, the benefits of using a biomarker such as FLA – which is cheap and readily assessed (ELISA), stable at room temperature, and more specific than serologic inflammatory markers (CRP and ESR) – are quite promising (Gisbert et al., 2009). FLA, like FC, may provide complementary information to that derived using existing clinical disease activity indices, including the Pediatric Ulcerative Colitis Activity Index (PUCAI), in which disease activity is weighted based on a particular patient’s subjective impressions (Assche, 2011). While the utility of FLA has yet to be fully explored, a better understanding of this marker could further our understanding of IBD and promote the development of newer generations of non-invasive biomarkers for use in the
diagnosis and assessment of patients with IBD (Gisbert et al., 2009; Kane et al. 2003). While research has been done to evaluate the predictive value of fecal biomarkers on treatment response, need for surgery, and disease relapse, more testing will be required before FLA and fecal calprotectin can be used reliably in clinical practice (Abraham & Kane, 2012). Ultimately, the clinical validation of fecal biomarkers, used in conjunction with information provided by disease activity indices and past medical history, would enable physicians to more expediently assess patients with symptoms suspicious for IBD (Gisbert et al., 2007, Abraham & Kane, 2012). The use of fecal biomarkers may also facilitate and improve ongoing monitoring of disease activity and response to therapy in patients with UC and CD (Assche, 2011).
SPECIFIC AIMS

Current and past studies demonstrate that numerous inflammatory biomarkers exist for identifying a patient with IBD. More specifically, research into specific fecal biomarkers indicates that FLA appears to be a highly sensitive and highly specific marker of intestinal inflammation. Moreover, FLA has shown to be very useful in discriminating patients with IBD from those with IBS or healthy controls (Kane et al., 2003; Walker et al., 2007). Given the notion that FLA appears to be a useful marker for intestinal inflammation, we hypothesize that FLA levels are a useful determinant of disease activity in patients with IBD, specifically UC. Therefore the goal of this study is to determine whether serial FLA measurements are predictive of both the response to steroid therapy and the likelihood of a clinical relapse. These measurements will then be used in conjunction with PUCAI scores and endoscopic study results to identify the relationship between FLA levels and these more standardized tools of disease evaluation. More specifically, we will evaluate:

1. Whether the changes in quantitative FLA measurements predict the likelihood of response to either parenteral or oral steroid therapy
2. The relationship between FLA levels and existing diagnostic and clinical assessment tools (e.g. PUCAI and/or clinical lab inflammatory markers)
3. The feasibility of developing a composite predictive disease metric for IBD
The data collected in this study will further define the predictive utility of FLA levels as a noninvasive fecal biomarker for evaluation of disease activity, steroid therapy response, the likelihood of disease relapse, and the potential for either rescue therapy or surgical intervention in patients with UC.
METHODS

Study Population

Consecutive pediatric patients (aged \( \leq 21 \) years of age) with known or suspected diagnosis of UC were admitted to the Gastroenterology (GI) inpatient service at Boston Children’s Hospital for the management of their symptoms. The definition of UC, including disease subtypes (ulcerative proctitis, distal UC, or pancolitis) was determined using standard clinical, endoscopic, histological, and radiological evidence of disease. Patients with Crohn Disease or undetermined intestinal malignancies were not recruited for the study. Additionally, patients who were diagnosed with HIV and/or Hepatitis B or C were excluded from the study. Patients who refused to provide serial stool samples were also excluded from study participation.

Recruitment Methods

Patients were recruited using access of Cerner® Powerchart, a patient medical record program, in conjunction with clinical interactions among GI inpatient service physicians. Powerchart is one medical record program used by the Boston Children’s Hospital to securely track and store patient medical information. Following identification of potential study candidates, patients were approached on the GI inpatient floor regarding study participation. Following explanation of the details of the study and the role of the subjects, both subjects and parents/guardians were given an opportunity to ask questions. If parents and patients remained interested in participating, informed consent
and assent of the participant and parents/guardians were obtained, respectively. Enrolled subjects were assigned a study designation number to maintain anonymity.

**Sample Collection and Processing**

Patients were asked to provide serial stool samples during their stay in the hospital and were also given the option to provide further samples following discharge. Stool samples were collected daily, the first one typically within 12 hours of admission and the final one upon patient discharge from the hospital. While daily samples were not used for study purposes, the procedure was implemented to facilitate sample collection for the nursing staff. The samples that were collected by nursing staff were then stored in a 4°C Celsius refrigerator. The most relevant samples for this study were collected on days 1 (baseline), 3, 5, and discharge. Collected samples were divided into three 2-mL aliquots, labeled with the appropriate information, and stored in a -80°C Celsius freezer. Samples remained in this freezer until they were ready for batch shipment to our testing facility. Shipment involved only one sample aliquot, leaving two in the freezer, in the event that samples were lost or needed to be retested at a later time point.

Patients agreeing to participate in the outpatient portion of the study were asked to provide stool samples every 14 days for an additional 12 weeks or until the end of the patient’s steroid therapy course. Patients were provided with the proper materials to collect and store stool samples prior to mailing. For outpatient sample processing and storage, an identical procedure to that of inpatient samples was followed.
Sample Testing

Batched stool samples were shipped to TechLab Inc. ® (Blacksburg, Virginia) who sponsored this study. TechLab Inc. ® personnel were blinded to disease activity. Two FLA tests developed by TechLab Inc. ®, IBD-SCAN™ (quantitative) and IBD-CHEK™ (qualitative), were performed on and results were determined for the fecal specimens following a 1:20 dilution in the kit. IBD-SCAN™ quantifies FLA in micrograms/milliliter (µg/mL). A reading of greater than or equal to 7.25 µg/mL was defined as an elevated FLA level, while values less than 7.25 µg/mL were considered baseline (normal). IBD-CHEK™ confirms the presence of FLA in terms of optical density (OD) at 450nm/620nm on a dual wavelength spectrophotometer. A reading of greater than or equal to .160 was defined as a positive result for stool samples. (TechLab®, 2008).
Case Report Forms

Case Report Forms (CRF) were completed for each patient that was enrolled in the study and provided at least one stool sample. CRFs were designed to expedite collection of relevant patient information from the electronic medical record and to facilitate data entry and analysis.

Inpatient CRFs include nine sections. Data in sections A-I are collected on the first day, while data for E-I are collected for every day that the patient is in the hospital. Sections A-D include general information, demographic information, patient disease information, and admission information, respectively. Figure 1, on the following page, provides a more detailed illustration of patient medical information (including diagnosis) necessary for the conduct of the study.
SECTION C: DISEASE INFORMATION

C1. Date of Diagnosis: __ __ / __ __ / __ __ __ __

C2. Montreal Classification
   Disease Extent:
   Ulcerative proctitis............1
   Distal UC..........................2
   Pancolitis.........................3

SECTION D: ADMISSION INFORMATION

D1. Date of Admission: __ __ / __ __ / __ __ __ __

D2. Reason for Admission:
   Diagnosis.................................................1
   Disease Flare...........................................2

D3. Date of Onset of Symptoms or Flare: __ __ / __ __ / __ __ __ __

D4. Prior Surgical Treatment:
   Yes...........1
   (If 0, skip to E1)
   No............0
   D4a. Date: __ __ / __ __ / __ __ __ __
   D4b. Type:
   Ileocecal Resection.................................1
   Strictureplasty.........................................2
   Right Hemicolectomy...............................3
   Left Hemicolectomy.................................4

Figure 1: Disease Information and Admission Information in the Case Report Form. Sections C and D from the CRF include details of each patient’s disease classification, date of diagnosis (based on colonoscopy results), reason for hospital admission (along with the start date of symptoms), and any prior GI surgical treatments. While most patients are admitted for disease flares, undiagnosed patients were also included in this study.
Among the data that were collected daily along with stool samples were PUCAI (Section E) scores, fecal lab levels, and patient blood lab values that corresponded to that day’s stool sample. Figures 2 and 3, on the following pages, provide a detailed view of the sections that were used to evaluate a patient’s disease activity during his/her stay in the hospital.
**SECTION E: ACTIVITY INDEX SCORES**

**E1. PUCAI Score:** __ __

**E2. PUCAI Components**

- E2a. Abdominal Pain: __ __ __ __ __
- E2b. Rectal Bleeding: __ __ __ __ __
- E2c. Stool consistency of most stools: __ __ __ __ __
- E2d. Number of stools per 24 hours: __ __ __ __ __
- E2e. Nocturnal Stools: __ __ __ __ __
- E2f. Activity Level: __ __ __ __ __

**E3. Disease Severity (circle 1):** Remission    Mild UC    Moderate UC    Severe UC

---

**SECTION F: FECAL LAB LEVELS**

**Stool Collected?** Yes... __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __
### SECTION UU: Discharge Outcome (Yes\(^1\)/No\(^0\))

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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</tr>
</thead>
<tbody>
<tr>
<td>2a. Steroids</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2b. Immunomodulators (Specify:______)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2c. Biologics (Specify:______)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2d. ASA (Specify:______)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2e. Antibiotics (Specify:______)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2f. Tacrolimus (Specify:______)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2g. Surgical Intervention (Date of procedure:________<strong>; Type of Procedure:</strong>____)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 3: Patient Discharge Outcome in the Case Report Form** – Section UU includes the patient’s discharge medications/interventions following his/her hospital visit.
Statistical Analysis

Data was collected and patient outcome was dichotomized into two populations: those that were medically responsive and subsequently discharged on corticosteroids and those that had medically refractory disease and required rescue (tacrolimus/infliximab) therapy. These two populations were then compared with respect to FLA levels (IBD-SCAN™). For both of the populations, the mean, standard deviation, median, and interquartile ranges were calculated. Appropriate parametric (t-test) and non-parametric (Wilcoxon rank-sum test) statistical testing was then used to determine statistical significance of the differences in percent change from day 1 to day 3 of hospitalization between the two groups of patients with respect to FLA levels (IBD-SCAN™).
RESULTS

Patient Population

A total of 19 patients diagnosed with UC provided informed consent prior to participation in this study. As seen in Figure 4, 10 of the 19 patients provided samples from at least the first three days of their hospitalization while the remaining 9 did not have sufficient sample provision to allow for analysis. Comparison of fecal and biochemical data collected on the day of admission and Day 3 of the hospital stay were then used for analysis.

Figure 4: Number of Patients and Sample Provision. 19 total patients (red bar) were enrolled in this study following provision of at least one stool sample. Of these 19, 10 were analyzed based on samples provided from day of admission and day 3 of hospitalization (yellow bar). 9 were excluded from the analysis due to insufficient sample provision during their hospital courses (blue bar).
We evaluated FLA levels as a prognostic outcome measure (discharged on steroids vs. need for rescue therapy). As seen in Figure 5, 3 patients were discharged on steroid therapy while 7 were discharged on rescue therapy (e.g. tacrolimus or infliximab).

![Figure 5: Discharge Outcome of Analyzed Patients.](image)

For the purpose of this study, predictive value of FLA was based on the IBD-SCAN™ values, since this assay has been previously validated as a continuous measure.

**Fecal Lactoferrin (IBD-SCAN™) and Discharge Outcome**

A t-test was performed to distinguish whether FLA levels (IBD-SCAN™) would prognose discharge disposition. Values including the mean, standard deviation, median,
and interquartile range for both patient groups (steroid therapy or rescue therapy) on both
days is provided in Table 2. Additionally, Figures 6 and 7 provide a graphical
representation of the data for day of admission and day 3 of hospitalization, respectively.
Patients that were discharged on steroids had median FLA levels on admission (653.3
µg/mL) that were almost 10-fold greater than values of patients put on rescue therapy
(96.5 µg/mL). However, by day 3, median FLA levels for both patient populations were
statistically indistinguishable (310.0 µg/mL and 321.7 µg/mL, respectively)

**Table 2: Distribution of FLA Levels for Two Patient Groups in µg/mL.** 10 patients
had stool samples analyzed for FLA levels (IBD-SCAN™) and were segregated into one
of two groups. 3 patients were categorized as discharged on steroids and 7 patients were
categorized as discharged on rescue therapy. The differences in values are displayed
above, however, no statistical significance was found. *p = 0.51

<table>
<thead>
<tr>
<th></th>
<th>Day of Admission</th>
<th></th>
<th>Day 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td>N</td>
</tr>
<tr>
<td>Discharged on steroids</td>
<td>3</td>
<td>662.3 (605.0)</td>
<td>653.3 (61.8,127.17)</td>
<td>3</td>
</tr>
<tr>
<td>Rescued with Tacrolimus/infliximab</td>
<td>7</td>
<td>307.5 (391.1)</td>
<td>96.5 (81.7,543.3)</td>
<td>7</td>
</tr>
</tbody>
</table>
Figure 6: Day of Admission Distribution of FLA Levels for Two Patient Groups. This chart is a graphical representation of the values listed previously in Table 2 for day of admission. IBD-SCAN™ values are listed on the y-axis (μg/mL). The ranges of these values in the two populations appear similar. However, differences between the median and IQR values for the two populations were not found to be statistically significant (p = 0.51)
Figure 7: Day Three Distribution of FLA Levels for Two Patient Groups. This chart is a graphical representation of the values listed previously in Table 2 for day 3 of hospitalization. IBD-SCAN™ values are listed on the y-axis (µg/mL). As seen in the diagram, the range of values between the two groups is quite different. However, the median and IQR values for the two populations are found to be similar. Once again, the differences were not found to be statistically significant (p = 0.51)
Using the above data, the percent change in FLA levels for both populations was calculated in order to determine whether trends could be found. Figure 8 provides a visual representation of the trend for each patient during their respective hospital stay.

**Figure 8: Trend in FLA Levels for Two Patient Groups.** This chart is a graphical representation of the changes in FLA levels for each of the 10 patients analyzed for the study from day of admission to day 3 of hospitalization. Patients discharged on steroids demonstrated an overall decline in levels from day 1 to day 3 (red lines). Patients discharged on rescue therapy demonstrated a range of outcomes for their hospital stays (yellow lines). IBD-SCAN™ values are listed on the y-axis (µg/mL).

These changes were used to calculate the total percent change for each patient. Results are shown in Table 3 and described graphically in Figure 9. The data revealed that patients that were medically responsive experienced a drop (-64.4%) in FLA over the first three days of their admission, while those that ultimately required rescue therapy
experienced an increase (+203.8%) in FLA. The changes in mean FLA levels approached but did not reach statistical significance (*p = 0.18).

**Table 3: Percent Change in FLA Levels of Two Patient Groups.** This table illustrates the differences in percent change between the two patient populations. Patients discharged on steroids demonstrated a negative percent change. Patients discharged on rescue therapy demonstrated an overall increase in percent change. (*p = 0.18)

<table>
<thead>
<tr>
<th>Percent change between admission and Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>
Figure 9: Percent Change in FLA Levels of Two Patient Groups. This chart is a graphical representation of total percent changes (day of admission to day 3) in FLA levels for both patient populations analyzed. Patients discharged on steroids demonstrated an overall percent change of -64.4% (blue bar). Patients discharged on rescue therapy demonstrated an overall percent change of +203.8% (yellow bar).

A generalized linear model and two-sample t-test were used in order to predict the change in FLA levels between day of admission and day 3. Since only two variables were assessed, the tests yielded the same results. There was a difference between the percent changes in FLA levels for the two populations. Yet, the effect of change in FLA levels did not quite reach statistical significance (p = 0.18).
DISCUSSION

Inflammatory Bowel Disease (IBD) occurs in children and adults throughout the world. While the causes of the disease are not yet identified, much effort has been applied to disease etiology, both from a genetic and environmental standpoint (Benchimol et al., 2011; Binder, 1998; Saeed & Kugathasan, 2008). Similarly, ongoing studies are centered on efforts to develop less invasive and more reliable ways to identify and manage patients with these disorders. To this end, the development of improved fecal biomarkers may contribute to a model of care that is less invasive, more cost effective, and more time efficient (Kane, Sandborn, Rufo et al., 2003; Assche, 2011).

While it is widely accepted that biomarkers such as Fecal Lactoferrin (FLA) and Fecal Calprotectin (FC) are useful in discriminating between both affected and unaffected patients (Abraham & Kane, 2012; Masoodi et al., 2011), as well as active and inactive disease states, there is a premium on incorporating these measure in diagnostic and prognostic algorithms. At this stage, neither differentiation of disease type (i.e. CD versus UC) nor the ultimate disease outcome can be reliably evaluated using fecal biomarkers (Abraham & Kane, 2012). Relatively little research has been conducted in order to determine the predictive utility of FLA levels in regards to treatment outcome, specifically while patients are hospitalized. The ability to use fecal biomarkers to quickly gather information about which treatment methods a patient will require while in the hospital has important implications. Among the most notable, patient care can be
implemented much more rapidly and therapy upon discharge will be more easily elucidated. This project hopes to begin to uncover relationships related to this goal.

Based on our data, certain observations were made that might indicate usefulness of FLA levels (IBD-SCAN™) in predicting clinical course and ultimately discharge disposition of hospitalized patients. In comparing the two patient populations (medically responsive vs. those requiring rescue therapy), it was found that the median FLA values measured on the day of admission were quite different despite having similar ranges of values (steroid outcome = 653.3 μg/mL; rescue outcome = 96.5 μg/mL) but trended towards being quite similar upon day 3 of hospitalization (steroid outcome = 310.0 μg/mL; rescue outcome = 321.7 μg/mL). The differences in values, however, were not found to be statistically significant (p = 0.51).

Interestingly, the change in FLA levels recorded on the day of admission and on hospital day 3 appeared to be different between the two populations. Our data indicated that patients discharged on steroids (n=3) showed a negative percent change (-64.4%) in FLA levels during the first three days of their hospitalization, while patients that required rescue therapy (n=7) by the time of discharge demonstrated an increase in their FLA levels (+204.8%). While the differences in mean values did not reach statistical significance (p = 0.18), this was a promising result: patients hospitalized for active disease and displaying decreasing FLA levels by hospital day 3 were more likely to be discharged on steroids, while those who showed stagnant or up-trending FLA levels were more likely to require more aggressive rescue therapy, such as tacrolimus or Infliximab.
The most important aspect of these findings is that it provides support for identifying and creating a specific objective measure for disease activity. Physicians currently rely on numerous metrics, both subjective (e.g. PUCAI) and objective (e.g. endoscopy and serological inflammatory markers), but still encounter complications. While PUCAI has been accepted as a very effective evaluation of disease activity, minor changes in a patient's symptom report can have a drastic impact on their score, possibly triggering inappropriate changes in their care (Turner, et al. 2007). Similarly, serologic inflammatory markers (CRP or ESR) may be confounded by other systemic ailments, making their use in treatment or prognostic algorithms less reliable. Having the ability to use biomarkers specific to intestinal inflammation could provide doctors with more concrete and objective evidence on which to base their clinical impression and changes in therapy. Our data might substantiate this claim since longitudinal sample collection was executed. This study design enabled the use of each patient as his/her own control in regards to FLA levels. Rather than relying on absolute FLA measurement, the ranges of which may differ from patient to patient, physicians can instead measure the change in FLA for individual patients. This personalized approach is likely to optimize medical decision-making and streamline not only clinical care but also expedite future intervention trials.

**Future Directions**

Based on these encouraging preliminary findings, the Center for Inflammatory Bowel Diseases at Boston Children’s Hospital will look to expand the sample size.
While such efforts were made shortly after study initiation by increasing the age range for inclusion criteria (initially: 18, now: 21), recruiting more patients and obtaining daily samples for 3 or more days will be of utmost importance. Related to sample collection, we will also attempt to collect samples and biochemical data over a greater length of time, in hopes that data collected on admission may provide more prognostic information in relation to patient disposition at discharge.

In regards to developing a more objective measure for prediction of discharge outcome, the potential for creating a composite measure of disease activity could prove extremely useful in substantiating medical treatment decisions. Such composite scores could involve the incorporation of serologic measures (e.g. ESR, CRP, Albumin) with measured FLA levels. By including these systemic markers with the colon-specific marker, disease activity and therapy outcome could be bolstered, mainly due to the presence of other objective measures of disease activity. Another measure to possibly incorporate with FLA levels could be PUCAI scores. However, such measures are subjective and the validity may be limited by patient report and developmental stage.

The findings of this thesis and this research overall have positive implications for management of patient care with respect to IBD, specifically UC. Studies of a larger scale that choose to analyze the predictive utility of FLA levels with respect to disease course and therapy outcome could prove to be quite valuable for both short-term and long-term treatment of patients with IBD.
REFERENCES


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Education: Bachelor’s of Science, Human Biology, 2012
University of California, San Diego

Candidate for Master’s of Science in Medical Sciences, May 2014
Boston University School of Medicine, Graduate Medical Sciences

Relevant Course Work: Biochemistry and Cell Biology, Cellular Organization of Tissues, Advanced Human Physiology, Pathology

Healthcare/Laboratory Work Experience:

Research Coordinator, Boston Children’s Hospital (Boston, MA), Department of Gastroenterology and Nutrition Center for Inflammatory Bowel Diseases: Dr. Paul A. Rufo (May 2013-Present)

- Aid in conduction of research to determine new ways to help physicians diagnose and monitor patients with inflammatory bowel disease, without relying on expensive, invasive testing, through the use of biomarkers
- Recruit patients and obtain informed consent for participation in studies in the hospital inpatient floor
- Maintain database and hard copies of study records
- Handle and process blood and stool samples
- Assist in study design and compose IRB protocols
Lab/Research Assistant, UCSD School of Medicine (San Diego, CA), Physiology Dept., Dr. Peter Wagner and Dr. Susan Hopkins (January -July 2012)
• Assist in preparation of protocol and submission for Institutional Review Board approval of a clinical study
• Assist in conducting exercise performance experiments for academic and medical research
• Operation of ParvoMedic metabolic cart system for maximal and sub-maximal VO2 performance tests
• Collected and analyzed blood samples for specific metabolites
• Cleaned and maintained lab facilities for efficient lab activity

Volunteer, UCSD Medical Center (San Diego, CA), Emergency Department (April 2012-August 2012)
• Assist nurses and technicians with daily tasks (e.g. room cleaning, patient meal delivery, patient transport)
• Stock IV and suture carts of ED with necessary items
• Transport samples to chemistry labs for analysis
• Interact with patients in ED rooms and waiting areas

Laboratory Assistant, Alta/Intertek Analytical Laboratories, San Diego, CA (June 2010-August 2010)
• Set up and running daily Enzyme-linked immunosorbent assays (ELISAs) in a GLP compliant laboratory environment supporting pharmaceutical research. Buffer preparation and glassware.

Laboratory/Administrative Assistant, Taligen Therapeutics, Cambridge, MA (June 2009-Aug. 2009)
• Helped organize and implement the set-up of a new on-site laboratory, including equipment and laboratory supply purchasing and set up of environmental health and safety
• Market research and competitive pipeline assessment of new drugs in clinical development

General Work Experience:

GMS Human Physiology Course Tutor, BU School of Medicine Graduate Medical Sciences, Boston, MA (September 2013-January 2014)
• Assist students in gaining a better understanding of concepts related to: Cell Physiology, Cardiovascular Physiology, and Gastrointestinal Physiology
• Provide both test-taking and study strategies for the topics covered
Recreational Summer Rowing Program Instructor, Boston University FitRec Center, Boston, MA (May 2013-August 2013)
- Instruct patrons of all rowing skill levels in the fundamental and advanced technical aspects of the sport of rowing
- Prepare advanced rowers for competitions related to the sport

UCSD Sports Facilities Supervisor, UCSD Main Gym, La Jolla, CA (April 2009-Present)
- Monitor activities and usage of Main Gym and Natatorium facilities
- Help set-up events both inside and outside of facility

Skills/Qualifications:
- Proficiency with Microsoft Word, Excel, PowerPoint, SPSS Data Entry and Data Builder, REDcap
- Knowledge of basic laboratory procedures
- Familiar with FDA Quality Compliance Procedures and trained specifically in Good Laboratory Practices and Good Clinical Practices
- Extensive leadership experience including: participation in Athletics-wide leadership workshops at UCSD, Vice-President of Student-run Intercollegiate Athletics Committee, and project coordination for Riverside Boat Club events and fundraisers)
- Exceptional teamwork and cooperation skills

Extracurricular Activities:
Riverside Boat Club (Provisional Member) (2013-present)
Event and Facilities Committee Member/Contributor
UCSD Men’s Varsity Rowing Team (2008 - 2012)
Varsity Captain (2012)
UCSD Master’s Swimming (2008 - 2012)
Triton Athletes Council (Vice-President) (2009 - 2012)

References Available Upon Request