Serial fecal ASCA measurements in the evaluation of children with Crohn's disease

https://hdl.handle.net/2144/16025

Boston University
SERIAL FECAL ASCA MEASUREMENTS IN THE EVALUATION OF CHILDREN WITH CROHN DISEASE

by

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B.A., New York University, 2012

Submitted in partial fulfillment of the requirements for the degree of
Master of Science

2015
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ACKNOWLEDGMENTS

Working at Boston Children’s Hospital has been a tremendous experience. Not only was I able to complete my master’s thesis at a world-class institution, but also was given learning opportunities that will inspire and inform my career as a future physician. I would like to thank my mentor Dr. Paul Rufo who taught me about what it is to be a dedicated physician and scientist. I also greatly appreciate the support and encouragement from my colleagues at the Center for Inflammatory Bowel Disease.

I would like to thank Dr. Simon Levy for his time and advice over the past two years. With Dr. Levy’s thoughtful guidance, I have challenged myself as a MAMS student and successfully taken the next step towards my goal of becoming a physician.

My mom, dad, and sister have always been a constant source of unyielding support and motivation, and I would not be where I am without them.
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RACHEL P. MOJDEHBAKHSH

ABSTRACT

Background: Pediatric patients with Inflammatory Bowel Disease (IBD) undergo costly and invasive investigations to diagnose and treat their chronic disease. To that end, it is important for researchers and physicians to continue to work to find novel tools to improve diagnosis and treatment processes. One of the main challenges is differentiating between the two main forms of IBD, Crohn disease (CD) and ulcerative colitis (UC). Physicians currently rely on a combination of endoscopic evaluations, mucosal biopsies, radiology studies, and biochemical testing to assess for the presence and extent of inflammation in the gastrointestinal (GI) tract. Serologic biomarkers can be useful to some extent, but changes in these markers do not typically reflect disease specific to the GI tract, or the state of inflammation related to a patient’s IBD. In contrast, fecal biomarkers have the unique potential to provide specific information about inflammation in the GI tract. While serum antibody levels have been well studied for use in the diagnosis of patients with IBD, fecal antibody levels and anti-saccharomyces cerevisiae antibody (ASCA) in particular, have not been extensively evaluated. In this study, we will assess the dynamic range of fecal ASCA levels in acute and convalescent fecal samples collected from children and adolescents with CD and UC.
Methods: We recruited pediatric patients from inpatient and ambulatory settings at the Gastroenterology Program at Boston Children’s Hospital. Patients had a diagnosis of either CD or UC. We collected baseline stool samples during a point of active disease, and follow-up samples three to six months later during a point of inactive disease. Samples were analyzed for fecal ASCA as well as lactoferrin (FLA), another marker of inflammation that can be measured in the stool.

Results: In patients with CD, fecal ASCA levels were significantly higher during active disease than during inactive disease. Additionally, fecal ASCA levels were higher in patients with CD than in patients with UC, regardless of disease activity. When compared to FLA, ASCA was shown to differentiate between CD and UC, with greater changes in the level of fecal ASCA (active – inactive) correlating with a diagnosis of CD. In patients with CD, FLA levels were significantly higher in the context of active disease than in inactive disease. However, FLA did not differentiate between CD and UC.

Conclusions: Our results suggest that fecal ASCA may be a new marker of inflammation in the GI tract. Unlike FLA, changes in fecal ASCA levels appear more dynamic in patients with CD. Future studies are required to further demonstrate both how changes in fecal ASCA may help physicians distinguish between different forms of IBD as well as how measurement of fecal ASCA may help assess disease activity and response to therapy in patients with CD.
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LIST OF ABBREVIATIONS

ASCA ............................................................ Anti-Saccharomyces Cerevisiae Antibody
ANCA ............................................................ Anti-Neutrophil Cytoplasmic Antibody
CD ...................................................................... Crohn Disease
CDL ..................................................................... Crohn-Like Disease
CRF ...................................................................... Case Report Form
CRP ...................................................................... C-Reactive Protein
CT ...................................................................... Computerized Tomography
DC ...................................................................... Dendritic Cell
ELISA ............................................................... Enzyme-Linked Immunosorbbent Assay
ESR ...................................................................... Erythrocyte Sedimentation Rate
FLA ...................................................................... Fecal Lactoferrin
GALT ................................................................. Gut-Associated Lymphoid Tissue
GI ...................................................................... Gastrointestinal
HCG ...................................................................... Human Chorionic Gonadotropin
IBD ...................................................................... Inflammatory Bowel Disease
IBS ...................................................................... Irritable Bowel Syndrome
MALT ................................................................. Mucosa-Associated Lymphoid Tissue
MRI ...................................................................... Magnetic Resonance Imaging
OD ...................................................................... Optical Density
UC ...................................................................... Ulcerative Colitis
INTRODUCTION

Inflammatory Bowel Disease (IBD) is characterized by idiopathic and chronic inflammation of the gastrointestinal (GI) tract (“Inflammatory bowel disease,” 2015). IBD encompasses two main diagnoses, Crohn disease (CD) and ulcerative colitis (UC), and the two are distinguished by the location and extent of inflammation (“Crohn’s Disease,” 2014; “Ulcerative Colitis,” 2014). IBD affects over one million Americans and at least 20% of those are diagnosed in childhood (Hendrickson, Gokhale, & Cho, 2002; Rufo & Bousvaros, 2006). Studying early onset disease in children may provide researchers with a unique opportunity to obtain new insights into the pathogenesis of IBD (Sauer & Kugathasan, 2010). Presently, the process for diagnosing IBD is both invasive and costly and relies on endoscopic assessment and the interpretation of mucosal biopsies. Research is underway to find novel and less invasive approaches to both stratify which patients, and especially which children, are most likely to benefit from more definitive testing and to assess an individual patient’s response to therapy. One way researchers are working to solve this problem is with the use of fecal biomarkers. These proteins can be measured in the stool of patients with IBD, and previous studies have demonstrated that they may be useful in assessing the presence of inflammation in the GI tract (Assche, 2011).

Biomarkers

Biomarkers are readily obtained objective indicators of relevant biological outcomes occurring in the body. Ideally, changes in levels of biomarkers correlate with a
clinically relevant endpoint. A biomarker can be anything from blood pressure to an antibody level. The use of biomarkers becomes necessary when measuring an outcome directly is too invasive, costly, or cannot be sustained. Changes in the level of a particular biomarker may predate changes in clinical course. As such, the use of biomarkers may afford clinicians the opportunity to provide proactive changes in therapy, thereby reducing the likelihood of the development of clinically significant disease. Therefore, biomarkers can be used to stratify clinical outcome or response to a particular therapy (Strimbu & Tavel, 2011).

Some examples of current biomarkers in widespread use include serum cholesterol for risk of heart disease and human chorionic gonadotropin (hCG) for detection of a pregnancy in a urine test. To clinicians, biomarkers are useful for making a diagnosis, assessing response to therapy, predicting relapse, and assessing disease state. In addition, biomarker development is of tremendous interest to the pharmaceutical industry in that robust biomarkers can be employed to reduce both the cost of clinical drug trials and potential adverse effects. Biomarkers can also elucidate disease processes and indicate potential targets for new interventions.

Biomarkers, therefore, can have an impact upon the entire spectrum of healthcare, from the creation of drugs to the long term care of patients. However, biomarkers must be validated to demonstrate that they accurately reflect underlying processes and provide valid and relevant information about a condition if they are to dictate subsequent actions. In addition, it is important to remember that although biomarkers may serve to reflect changes in biological processes, they are not the actual outcome in question.
Consequently, physicians and researchers must be aware of this limitation when drawing conclusions about changes in therapy and reporting clinical prognosis (Strimbu & Tavel, 2011).

**Clinical Presentation of Crohn Disease and Ulcerative Colitis**

Patients presenting with CD and UC can look very similar clinically. Ultimately, physicians must rely on the location and histology of the inflamed tissue in the context of patients’ symptoms to make a definitive diagnosis. CD can affect any portion of the GI tract and the involved segments can be discontinuous. In contrast, the inflammation observed in patients with UC is confined to the colon and is continuous in nature. Additionally, the inflammation in patients with CD can be transmural, affecting multiple layers of the GI tract, whereas the inflammation seen in UC is generally limited to the superficial mucosa lining the colon (Hendrickson *et al.*, 2002; Abraham & Cho, 2009). Due to the invasive nature of inflammation in CD, patients are at risk for a number of long-term complications including strictures, obstructions, and fistulae or aberrant connections of the intestines to other portions of the GI tract or intestinal viscera (Abraham & Cho, 2009). Pediatric patients with CD typically present with disease limited to the ileocolonic or colonic regions. The majority of pediatric patients with UC are found to have pancolitis, which affects the entire colon, as opposed to primarily left-sided colitis that is more frequently found in adults with UC (Sauer & Kugathasan, 2010).
Patients with CD and UC can present with both intestinal and extraintestinal symptoms. The most common clinical symptoms are diarrhea, abdominal pain, and loss of appetite. The physical appearance of stool is one way to begin to differentiate between CD and UC. Patients with UC typically present with visible blood and mucus in their stool, which is not always the case in patients with CD (Hendrickson et al., 2002; Bousvaros et al., 2007). In contrast, patients with CD more often present with more indolent symptoms including weight loss and growth delay due to chronic inflammation in the small bowel, leading to intestinal dysfunction and malabsorption (Hendrickson et al., 2002).

Epidemiology of Inflammatory Bowel Disease

The incidence of IBD is highest in North America and Europe. However, IBD is becoming increasingly common in developing regions of the world including China, South Korea, India, Lebanon, Iran, Thailand, French West Indies, and North Africa (Ponder & Long, 2013). This increase in prevalence likely correlates with an intermingling of genetics and the “Westernization” of these countries with respect to diet and lifestyle, and thus provides some evidence for the role of environmental risks in the development of IBD (Loftus, 2004; Benchimol et al., 2011; Ponder & Long, 2013). Interestingly, emigrants moving from a geographic area with lower incidence to one with higher incidence ultimately are at greater risk for the development of IBD (Benchimol et al., 2011; Ponder & Long, 2013).
Age and gender are demographic factors that have an impact on the incidence and type of IBD. Relative to adult patients with IBD, there seems to be a higher incidence of CD than UC in pediatric patients, with a ratio of 2.8:1. Furthermore, there is a male to female ratio of 1.5:1 in children with CD, as compared to pediatric UC in which the ratio has been observed to be closer to 1:1. In contrast, there does not seem to be as clear an effect of gender on the incidence and prevalence of IBD in adult studies (Sauer & Kugathasan, 2010).

**Etiology of Inflammatory Bowel Disease**

IBD is thought to be a result of a confluence of genetic and environmental factors, though the exact relationship is unknown (Ponder & Long, 2013). Numerous genome wide association studies have identified more than 160 loci associated with susceptibility to IBD. Studies have shown that 5.7 to 15.5% of patients with UC have a first-degree relative with UC (Cioffi, 2015). Monozygotic twin studies have found a concordance rate of 50% for CD and 19% for UC (Ponder & Long, 2013). Another study reported a 16-36% concordance rate in monozygotic twins (Benchimol et al., 2011). These findings clearly leave room for other factors to play a significant role in the development of IBD. There is now growing evidence to suggest that genetics play a more important role in early-onset pediatric IBD, partly because these patients have had less exposure to other potential environmental factors (Sauer & Kugathasan, 2010).

Common environmental risk factors that have been studied in the pathogenesis of IBD include breast-feeding, smoking, diet, and antibiotic use (Loftus, 2004; Ponder &
The effects of breast-feeding on the development of IBD are somewhat unclear. Most studies point to a protective role of breast-feeding against the development of IBD. However, it may be more important to look at the duration of breast-feeding. A more recent study found a protective effect only after a duration of at least three months (Ponder & Long, 2013; Cioffi, 2015). Smoking increases the risk of developing CD. However, there is an inverse relationship between active smoking and the risk of developing UC. In contrast, ex-smokers are 70% more likely to develop UC than people who have never smoked (Loftus, 2004; Ponder & Long, 2013). Studies implicating high dietary fat or sugar in the development of IBD have been inconclusive, though data suggest that diet does still seem to play some role (Loftus, 2004). There seems to be a clear connection between increased antibiotic exposure and a risk of developing IBD, especially when the antibiotics exposure occurred early in life. One retrospective study done in the UK compared the incidence of IBD in children, aged two and older, who either had been exposed to antibiotics or not. They found an 84% risk increase for developing IBD in those children who had been exposed to antibiotics. Furthermore, the risk decreased the older a child was and increased if a child took more than two courses of antibiotics. A Canadian study showed that if a child is exposed to antibiotics during the first year of life, the risk of developing IBD is increased by almost three-fold. However it remains unclear as to whether the effect of antibiotics is being mediated through direct effects on the intestinal microbiome or if antibiotic use is a marker for other predisposing factors directly related to the development of IBD (Ponder & Long, 2013).
Hypotheses related to risk factors associated with a Westernized life style have been proposed to explain the relationship between the environmental exposures and the development of IBD. The hygiene hypothesis suggests that the “cleaner” environments found in Western society are associated with higher prevalence of IBD, and that the greater prevalence of more harmful microbes in environments of less developed countries is associated with less IBD. One reason for this is because the interaction between the immune system and microbes fosters a regulated relationship between these two environments, allowing the immune system to learn to tolerate commensal bacteria while fighting harmful microbes. Therefore, when the immune system is exposed to fewer microbes, the immune system may develop improperly and as a result have an inappropriately severe response to commensal bacteria in the GI tract (Ponder & Long, 2013). In contrast, the “cold-chain hypothesis” suggests that refrigeration of our food has selected the bacteria we ingest, thus contributing to the development of IBD (Benchimol et al., 2011). Specific bacteria, known as psychotropic bacteria, that exist and thrive at lower temperatures can be found in CD patients and may contribute to IBD pathogenesis (Hugot, et al., 2012). The exact effects of these factors on the development of IBD remain elusive and are likely to be better understood only when ongoing research better elucidates genetic interactions and their effects on the microbiome.

In recent years, more attention has been paid to the intestinal microbiota and how alterations to it may lead to disease. The microbiota is acquired at birth but then undergoes many changes during the first few years of life. Figure 1 shows how the microbiota develops over time and what factors affect it. The microbiome encompasses
the genomic contents of the human microbiota, which is a community of microbes living within the human body. Newer studies have demonstrated that the interaction between the host immune system and the microbiota can be beneficial to the host, or can result in an apparent disequilibrium that can predispose toward the development of mucosal inflammation (Abraham & Cho, 2009). We know now that the microbiota plays an integral role in the development of the immune system, metabolism, and general homeostasis within the human body (Villanueva-Millán, Pérez-Matute, & Oteo, 2015).

**Figure 1: How The Intestinal Microbiome Develops.** Figure was taken from (Villanueva-Millán, Pérez-Matute, & Oteo, 2015).

When the microbiome is altered, or unbalanced, it is a known contributor to the development of disorders such as obesity, *C. difficile* infections, and IBD. Therefore, therapies with the intention of restoring the microbiome to a productive and balanced...
state are being investigated as a way to treat the resulting disorders. These therapies include prebiotics, probiotics, and fecal transplants (Villanueva-Millán, Pérez-Matute, & Oteo, 2015).

In the context of obesity, studies have shown that colonizing germ-free mice with the microbiome from obese mice makes the recipient mice obese. This highlights the effects of the microbiome on host metabolism and utilizing energy from food (Villanueva-Millán, Pérez-Matute, & Oteo, 2015).

Of the many ways the microbiome can be altered, antibiotic use is the most common (Villanueva-Millán, Pérez-Matute, & Oteo, 2015). Antibiotics kill bacteria in the GI tract, therefore changing the composition of the microbiota. This becomes especially relevant in the case of C. difficile infections. The hallmark of a healthy microbiome is diversity. Studies show that patients who develop a C. difficile infection after antibiotic use have a less diverse microbiome than that of patients without an infection. Therefore the less diverse and unbalanced microbiota affects the host immune system’s ability to identify and fight harmful microbes (Monaghan, 2015).

Additionally, because the microbiota is in constant contact with the immune system in the GI tract, changes to the microbiota can have an impact on how the immune system functions. In a healthy GI tract, the microbiota promotes an anti-inflammatory environment. When the microbiota becomes dysfunctional, the presence of harmful microbes can create a pro-inflammatory environment. This in turn causes further dysregulation of the microbiota perpetuating the evolution of inflammatory diseases like IBD (Maranduba et al., 2015).
Pathogenesis of Inflammatory Bowel Disease

The most extensive subset of our immune system lies within the mucosa lining the gastrointestinal, pulmonary, and genitourinary tissues and is referred to as mucosal-associated lymphoid tissue (MALT). This type of tissue interacts directly with the outside world and is therefore specialized in different parts of our body. For example, gut-associated lymphoid tissue (GALT) is found in the GI tract (“Mucosa-Associated Lymphoid Tissue,” 2014). GALT represents the largest group of lymphoid tissues in our body, consisting of mesenteric lymph nodes, Peyer’s patches, and individual lymphocytes (Forchielli & Walker, 2005). These structures monitor the epithelial barrier and are responsible for differentiating between the commensal microbiome and invading pathogens. Dendritic cells (DCs) in particular are responsible for selectively responding to potential pathogens while remaining immune tolerant to native microbiota that populate the gut (Tezuka & Ohteki, 2010).

In a healthy gut, immune homeostasis is dependent upon a bidirectional communication between the intestinal microbiota, the epithelium, and lymphoid tissue (Tezuka & Ohteki, 2010). In IBD, this balance can be disrupted through a pathological interaction between monitoring lymphoid cells and the microbiota. Additionally, when the epithelial layer is damaged or dysfunctional in some way, allowing excessive contact between the microbiota and lymphoid tissue, an inflammatory response can ensue. Defects in mucosal tight junctions between adjacent epithelial cells, as well as alteration in the mucus layer or secreted antimicrobial compounds, are examples of epithelial dysfunction that can predispose to inflammation. The ensuing inflammation can result in
ongoing epithelial injury, perpetuating the contact between the microbiota and lymphoid tissue and resulting in the chronic damage observed in the histologic findings from patients with IBD (Abraham & Cho, 2009).

**Diagnostic Techniques and Importance of Disease Differentiation**

It is difficult to differentiate between CD and UC based on clinical symptoms alone. Therefore, clinicians currently rely on a repertoire of biochemical tests, radiologic studies, and endoscopic evaluation with mucosal biopsies (Hendrickson *et al*., 2002). Radiologic tests, including MRI, CT scan and fluoroscopy, can be used to assess areas of the small intestine that cannot be reached using standard endoscopic procedures (Hendrickson *et al*., 2002). However, both CT scans and fluoroscopy expose the patient to radiation, which is more of a concern for pediatric patients, and MRI is very costly. In most cases, endoscopic and histologic studies can definitively differentiate between UC and CD. Making the diagnosis of CD becomes easier if there is obvious inflammation of the small bowel, perianal disease is apparent, or if granulomas (collections of immune cells) are observed in the biopsy. An endoscopist can usually see some differences on the mucosal surface, such as deep ulcers and discontinuous inflammation, which would be indications of colonic CD. Visual signs of UC include confluent erythema, tissue granularity, and loss of vascular pattern on the mucosa. Even with these distinctions, there are patients in whom the clinical and histologic pictures exhibit features of both CD and UC. In these cases, clinicians categorize these patients as having “indeterminate colitis” (Bousvaros *et al*., 2007).
The cost and logistics inherent in the present paradigm for IBD diagnosis continue to motivate physicians to find more reliable ways of screening for and assessing patients with IBD (Bousvaros et al., 2007). In children with IBD, preventing a substantial delay in diagnosis is vital as untreated inflammation can result in an increased risk for growth and pubertal delay, and this can have a long lasting effect into adulthood (Sauer & Kugathasan, 2010). Patients with UC usually present with acute symptoms, such as bloody diarrhea, sooner than those with CD. In these cases, the more indolent presentation of CD can lead to delayed diagnosis (Hendrickson et al., 2002). Additionally, though endoscopy/colonoscopy is the “gold standard” for diagnosis of IBD, the procedure requires bowel preparation and anesthesia making this a costly and invasive experience for pediatric patients (Assche, 2011). This is yet another incentive to continue to develop better and more efficient ways of diagnosing and differentiating IBD in a pediatric population.

**Serologic and Fecal Biomarkers in IBD**

Physicians are now looking to serologic and fecal biomarkers to more efficiently and less invasively diagnose and differentiate between different forms of IBD. Serologic biomarkers can be useful in detecting non-specific inflammation (Abraham & Kane, 2012). The most commonly used serologic biomarker used for the past 10-15 years is C-reactive protein (CRP). CRP is an acute-phase protein that is produced by the liver. In response to an inflammatory condition, CRP is produced by hepatocytes and released into systemic circulation. The sensitivity for CRP for differentiating between UC and CD
ranges from 50-60%. Serum CRP levels reflect inflammation anywhere in the body and are therefore too non-specific to be used to diagnose or monitor IBD activity (Assche, 2011; Cioffi, 2015). Another commonly used serologic biomarker for inflammation is erythrocyte sedimentation rate (ESR). Similar to CRP, an increased response in ESR is indicative of increased inflammation. ESR can provide insight into the changes in plasma protein composition during an inflammatory response (Cioffi, 2015).

Fecal biomarkers allow physicians to measure proteins that originate from the GI tract, making them specific to GI inflammation. Existing data suggest that fecal biomarkers can help determine the need for further testing, assess disease activity, and track a patient’s response to treatment. For example, when a differential diagnosis includes IBD and irritable bowel syndrome (IBS), a functional non-inflammatory syndrome, measurement of fecal biomarkers helps determine if a patient is likely to benefit from endoscopic evaluation (Abraham & Kane, 2012). With respect to assessing disease activity in IBD, fecal biomarkers of inflammation, including fecal lactoferrin (FLA) and calprotectin, have been shown to correlate with disease activity with higher levels indicating greater disease activity in patients with IBD (Assche, 2011; Abraham & Kane, 2012). Eliminating or reducing the need for endoscopic evaluation to assess disease activity in patients with IBD is important especially in the case of pediatric patients (Assche, 2011). Though fecal biomarkers appear to be more specific for assessing intestinal inflammation than those measured in the serum, it is important to note that existing fecal biomarker levels do not differentiate autoimmune (IBD) from infectious causes of inflammation in the bowel (Assche, 2011; Abraham & Kane, 2012).
Serum ANCA and ASCA

Two serologic biomarkers that have been associated with IBD are anti-neutrophil cytoplasmic antibody (ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA). ANCA levels have been shown to more closely correlate with UC than CD with rates of 20-85% and 2-28%, respectively. In contrast, serum ASCA levels tend to correlate with CD more than UC with rates of 39-69% and 5-15%, respectively (Cioffi, 2015). However, the sensitivity of ASCA drops when the patient has purely colonic CD. In distinguishing IBD from non-inflammatory conditions with similar symptoms, such as IBS, ASCA has a sensitivity of 31-45% and a specificity of 90-100% for diagnosing IBD (Prideaux, et al., 2012).

When these two biomarkers are used together, they yield a better diagnostic result than when used separately. Interestingly, one study showed that when reclassifying indeterminate colitis patients, a combined positive ASCA and negative ANCA predicted CD in 80% and a combined negative ASCA and positive ANCA predicted UC in 64% of these patients (Prideaux, et al., 2012).

Stability of serologic ANCA and ASCA are of importance when used in the context of a dynamic disease such as IBD. Serum ASCA levels appear to be stable over time in patients with CD and across disease activity. ANCA appears to also be stable over time in both UC and CD across disease activity. As such, these serologic biomarkers cannot be used to assess the level of disease activity in patients with IBD (Prideaux, et al., 2012). In general, though serologic biomarkers such as ANCA and ASCA have been shown to be of some use in differentiating CD from UC, the results are not reliable
enough to replace the endoscopic, histologic, radiologic, and other clinical means of diagnosis.

**Fecal Lactoferrin**

FLA is a protein derived from neutrophils, cells that play a role in the innate immune response. FLA arising from intestinal neutrophils is stable and is not degraded by bacterial enzymes in the small or large intestine, thereby making it a reliable marker of inflammation in the intestine (Assche, 2011). FLA measurements are useful in differentiating patients with active IBD *versus* inactive IBD. Studies have shown FLA levels may correlate to UC activity, as opposed to CD activity (Abraham & Kane, 2012). Consequently, though FLA provides valuable information regarding the general diagnosis of IBD, further studies are necessary to define the best approach to using this fecal biomarker in the diagnosis and interval assessment of patients with IBD.

**Fecal ASCA**

ASCA is thought to be produced in mesenteric lymph node, though its exact purpose is unknown (Reese *et al.*, 2006). Interestingly, studies have shown that the presence of ASCA does not seem to correlate with exposure to *S. cerevisiae*. Instead, the presence of these antibodies may not be indicative of increased permeability to microbes, but rather a decreased tolerance to microbes existing in our bodies (Prideaux, *et al.*, 2012). Like other serum antibodies, ASCA is also secreted into the stool and may be more informative as a fecal biomarker to both identify and assess disease activity in
patients with IBD. Almost no research has been done to date on the presence or utility of fecal ASCA in patients with IBD. One study was conducted comparing serologic and fecal ASCA against endoscopic findings in the context of assessing IBD patients who had surgical treatment for UC resulting in the creation of an ileal pouch. One common complication of this surgery is developing Crohn-like disease (CDL) of the pouch. The researchers found that in determining the cause of pouch disease, fecal ASCA was more useful than serum ASCA in determining whether the cause was CDL or other pouch disorders (Tang et al., 2012).

**OBJECTIVES**

Fecal biomarkers are an emerging area of research, and there are still many questions about which biomarkers are most accurate for use in assessing disease activity or differentiating between CD and UC. FLA and calprotectin have been shown to be useful in differentiating patients with inflammatory and those with non-inflammatory GI disorders. However, they offer little insight into discerning the nature (infectious vs. inflammatory) or type (CD vs. UC) of inflammation. ASCA has been shown to have higher specificity for CD and we would like to extend this line of research. Specifically, we will evaluate:

1. Whether fecal ASCA can differentiate between active and inactive CD in the same patient
2. Whether fecal ASCA can differentiate between CD and UC
3. How information provided by fecal ASCA compares to that provided by FLA
METHODS

Participants

For this study, 47 pediatric patients (aged 0-18 years) with IBD were included in our analyses: 35 with CD, and 12 with UC. Participants were patients at Boston Children’s Hospital. To be eligible for our study, patients had to carry an established diagnosis of either CD or UC. Patients with HIV, Hepatitis B or C, or those who had undergone a total colectomy were excluded from our study. Furthermore, if patients were unable to provide a stool sample, they were also excluded.

Recruitment

We determined the eligibility of patients using PowerChart® to view patients’ medical history. PowerChart® is an electronic medical records database. Patients meeting the eligibility criteria were recruited in both inpatient and ambulatory settings. Approval was obtained from the Institutional Review Board. Once the eligibility of a patient was confirmed, we approached the patient to explain our study objectives and requirements for participation. We acquired consent from both the patient and a parent and assigned the patient a study ID number. Only the study ID number was used from this point forward to identify any data. For inpatients, we placed a collection cup in their inpatient bathroom and for outpatients, we gave the patient a stool collection kit to take home and mail back to us with a sample. When patients provided a stool sample, they were reimbursed $10 for their participation. Three to six months after the baseline stool sample
was provided, patients were contacted to provide a follow-up sample and were again reimbursed $10 if a follow-up stool sample was provided.

Sample Processing and Testing

Immediately after sample collection, the samples were stored at 20 °C for no longer than one week. The stool samples were subsequently transferred from the sample cups into 2 ml tubes. Stool samples were handled under a chemical hood and transferred using pipets or wooden spatulas. The 2 ml tubes were labeled and placed in a freezer at -80 °C for storage.

Stool samples were batched and shipped overnight on dry ice to TECHLAB® (Blacksburg, VA) to be analyzed. To evaluate fecal ASCA, TECHLAB® used a commercially available assay, ASCA-CHEK. This enzyme-linked immunoassay uses antigens of ASCA to detect ASCA levels in feces. The ASCA-CHEK was run according to the TECHLAB® package insert (TECHLAB, Inc., 2008). To evaluate FLA, TECHLAB® used IBD-CHEK®, which is a qualitative ELISA. IBD-CHEK® was run according to the TECHLAB® package insert (TECHLAB, Inc., 2008). Both ASCA-CHEK and IBD-CHEK are qualitative assays and the results are reported in terms of optical density (OD). Technicians at TECHLAB® were blinded to all patient information except the sample collection date.
Case Report Forms

A case report form (CRF) was completed for each patient who provided a baseline stool sample. If patients provided a follow-up sample at a later time point, the follow-up portion of the CRF was also completed. CRFs included relevant medical history of the patients, including information about diagnosis, medications, labs, and imaging studies (Figures 2-4). Additionally, information about the treatment received in the hospital at the time the stool sample was collected was also recorded in the CRFs. CRFs were completed using medical records from PowerChart®.
### SECTION D: DISEASE INFORMATION

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D1. Diagnosis:</strong></td>
<td>CD.......................... 1</td>
</tr>
<tr>
<td></td>
<td>UC.......................... 2</td>
</tr>
<tr>
<td></td>
<td>Constipation............ 3</td>
</tr>
<tr>
<td><strong>D2. Date of Onset of Symptoms:</strong></td>
<td>__ __ / __ __ / __ __ __ __</td>
</tr>
<tr>
<td><strong>D3. Date of Diagnosis:</strong></td>
<td>__ __ / __ __ / __ __ __ __</td>
</tr>
<tr>
<td><strong>D4. Activity:</strong></td>
<td>Active........ 1</td>
</tr>
<tr>
<td><em>(If 2, Skip to D6)</em></td>
<td>Inactive...... 2</td>
</tr>
<tr>
<td><strong>D5. Reason (if active):</strong></td>
<td>Increase dose of existing medications........ 1</td>
</tr>
<tr>
<td></td>
<td>Add new medications to control symptoms.  2</td>
</tr>
<tr>
<td></td>
<td>New testing to diagnose symptoms............ 3</td>
</tr>
<tr>
<td></td>
<td>Missing.......................................... -9</td>
</tr>
<tr>
<td><strong>D6. Surgical Treatment:</strong></td>
<td>Yes.........1</td>
</tr>
<tr>
<td><em>(If 2, skip to E1)</em></td>
<td>No.........0</td>
</tr>
<tr>
<td><strong>D6a. Date:</strong></td>
<td>__ __ / __ __ / __ __ __ __</td>
</tr>
<tr>
<td><strong>D6b. Type:</strong></td>
<td>Ileocecal Resection............................. 1</td>
</tr>
<tr>
<td></td>
<td>Stricturesplasty.............................. 2</td>
</tr>
<tr>
<td></td>
<td>Right Hemicolectomy........................... 3</td>
</tr>
<tr>
<td></td>
<td>Left Hemicolectomy............................ 4</td>
</tr>
<tr>
<td></td>
<td>Colectomy and ileal anal pull through..... 5</td>
</tr>
<tr>
<td></td>
<td>Other (specify______________________)..... 6</td>
</tr>
</tbody>
</table>

Figure 2: Disease Information Recorded on CRF. Section D includes the diagnosis, date of diagnosis based on diagnostic colonoscopy, the state of disease based on patient’s current symptoms, and general treatment goals if patient has active disease. Additionally previous relevant surgical treatments are recorded.
**SECTION G: MONTREAL CLASSIFICATION**

**G1. Crohn Disease:**

- Yes………………..1
- No………………..0  *(if no, skip to G2)*

  **G1a. Age of Onset:**
  - <16y……………1
  - 17-40y…………2

  **G1b. Location:**
  - Ileal………………._
  - Colonic…………._
  - Ileocolonic……._
  - Upper GI……._

  **G1c. Behavior:**
  - Nonstricturing, Nonpenetrating……._
  - Strictures…………………………………._
  - Penetrating……………………………….._
  - Perianal disease modifier…………._

**G2. Ulcerative Colitis:**

- Yes……………..1
- No……………….0  *(if no, skip to H1)*

  **G2a. Extent:**
  - Ulcerative proctitis........1
  - Distal UC.........................2
  - Pancolitis.........................3

  **G2b. Severity:**
  - Remission......................1
  - Mild UC..........................2
  - Moderate UC....................3
  - Severe UC.......................4

---

**Figure 3: Disease Classification on CRF.** Section G includes specific information about the disease location and nature. This information is different depending on the diagnosis.
**SECTION L: BASELINE BLOOD WORK**

<table>
<thead>
<tr>
<th>CBC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L1. WBC</td>
<td>_ _ _ _ K cells/µL</td>
</tr>
<tr>
<td>L2. HCT</td>
<td>_ _ _ _ mg/dL</td>
</tr>
<tr>
<td>L3. PLT</td>
<td>_ _ _ _ mg/dL</td>
</tr>
<tr>
<td>L4. Neutrophils</td>
<td>_ _ %</td>
</tr>
<tr>
<td>L5. Lymphs</td>
<td>_ _ %</td>
</tr>
<tr>
<td>L6. Eos</td>
<td>_ _ %</td>
</tr>
<tr>
<td>L7. Monos</td>
<td>_ _ %</td>
</tr>
</tbody>
</table>

**Chemistry**

| L8. Albumin | _ _ g/dL |
| L9. SED Rate | _ _ mm/hr |
| L10. ASCA   | _ _ eu/mL |
| L11. ANCA   | _ _ eu/mL |
| L12. Amylase| _ _ unit/L |
| L13. ALT    | _ _ unit/L |
| L14. Bilirubin (Tot) | _ _ mg/dL |
| L15. Alkaline Phosphatase | _ _ unit/L |
| L16. CRP    | _ _ mg/dL  |

**SECTION M: BASELINE LEVELS**

| M1. IBD-CHEK™ Fecal Lactoferrin (OD450/620) | _ _ _ _ |
| M2. IBD-CHEK™ Interpretation | Positive………..1  
Negative………..0 |
| M3. IBD-SCAN™ Fecal Lactoferrin (µg/mL) | _ _ _ _ _ µg/mL |
| M4. IBD-SCAN™ Interpretation | Positive………..1  
Negative………..0 |
| M5. Fecal ASCA-CHEK™ (OD450/620) | _ _ _ _ |
| M6. Fecal ASCA-CHEK™ Titer | _ _ _ _ |
| M7. Fecal ASCA-CHEK™ Interpretation | Positive………..1  
Negative………..0 |
| M8. Serum ASCA-CHEK™ (OD450/620) | _ _ _ _ |
| M9. Serum ASCA-CHEK™ Titer | _ _ _ _ |
| M10. Serum ASCA-CHEK™ Interpretation | Positive………..1  
Negative………..0 |

**Figure 4: Serum and Fecal Lab Values on Day of Sample Collection.**

Sections L and M include serum and fecal lab values, respectively, on or around the same day. These values can be used to compare different types of inflammatory markers such as the serologic biomarker CRP and fecal biomarker ASCA.
RESULTS

Patient Population

Our analyses included 47 patients (35 with CD and 12 with UC) who provided both a baseline and follow-up sample. Active disease was defined as patients experiencing increased clinical symptoms, including diarrhea and abdominal pain. To address this, clinicians typically added a medication, changed the dose of an existing medication, or ordered diagnostic studies to better elucidate the nature of a patient’s increased symptoms. During an inactive disease state, patients were in clinical remission and were not experiencing clinical symptoms of their disease. Analyzing paired samples allowed us to evaluate fecal biomarker levels in both active and inactive disease within the same patient. We used non-parametric tests given our relatively small sample sizes.

Effect of Disease Activity on ASCA Levels

We first conducted a related-samples Wilcoxon signed rank test (“Wilcoxon Signed Rank Test,” 2013) comparing the fecal ASCA levels in active versus inactive CD and UC separately. We found that fecal ASCA levels were significantly higher (p = .001) in CD patients with active disease than in those with inactive disease (Figure 5). In contrast, in patients with UC, fecal ASCA levels were not significantly different (p = .859) in active and inactive disease states (Figure 6).
Figure 5: Effect of Crohn Disease Activity on ASCA Levels. This graph depicts ASCA levels on the y-axis (OD, optical density) and the CD activity state on the x-axis. ASCA levels are significantly higher in patients when their disease is active than when it is inactive. This analysis included 35 CD patients’ paired samples. (**p < .01)
Figure 6: Effect of Ulcerative Colitis Activity on ASCA Levels. This graph depicts ASCA levels in patients with active CD to those observed in patients with active UC. We repeated this test to compare ASCA levels in patients with inactive CD and inactive UC. We found that ASCA levels measured in patients with active CD are significantly higher ($p = .000$) than those observed in patients with active UC. Similarly, ASCA levels in patients with inactive CD are significantly higher ($p = .024$) than in inactive UC. This comparison shows that not only does ASCA reflect disease activity, but may also help to differentiate patients with CD from those with UC (Figure 7).
Figure 7: ASCA Levels Differentiate Between CD and UC. This graph depicts ASCA levels on the y-axis (OD, optical density) and both the disease and activity state on the x-axis. Here we compared active disease and inactive disease across CD and UC. ASCA levels are significantly higher in CD regardless of disease activity. (** p < .01, * p < .05)

Effect of Disease Activity on FLA Levels

We conducted a related-samples Wilcoxon signed rank test comparing the FLA levels in patients with active versus inactive CD and UC separately. We found that in patients with CD, FLA levels were significantly higher (p = .021) when their disease was active than when it was inactive (Figure 8). In contrast, in patients with UC, FLA levels did not discriminate between patients with active and inactive disease (p = .060, Figure 9).
Figure 8: Effect of Crohn Disease Activity on FLA Levels. This graph depicts FLA levels on the y-axis (OD, optical density) and CD activity state on the x-axis. FLA levels are significantly higher in active disease than in inactive disease within the same patient. This analysis included 35 CD patients’ paired samples. (*p < .05)
Figure 9: Effect of Ulcerative Colitis Disease Activity on FLA Levels. This graph depicts FLA levels on the y-axis (OD, optical density) and UC activity state on the x-axis. FLA levels are not significantly different in active disease and inactive disease in patients with UC. This analysis included 12 UC patients’ paired samples. (\( p > .05 \))

We conducted an independent-samples Mann-Whitney U test to compare FLA levels in active CD to active UC, then inactive CD to inactive UC. We found FLA levels in patients with active CD were not significantly different (\( p = .643 \)) from those measured in patients with active UC. Similarly, FLA levels in patients with inactive CD were not significantly different (\( p = .065 \)) those measured in patients with inactive UC. This data suggests that FLA is not a specific marker of disease activity and cannot be used to differentiate between patients with CD and those with UC (Figure 10).
Figure 10: FLA Levels Cannot Differentiate Between CD and UC. This graph depicts FLA levels on the y-axis (OD, optical density) and both disease and activity state on the x-axis. Here we compared active disease to inactive disease across CD and UC. FLA levels do not differ between CD and UC depending on disease activity. (*) p > .05

Overall Change in Fecal Biomarker Levels

To illustrate the overall decrease in fecal biomarker levels between the active and inactive disease states, we calculated the change (active – inactive) in ASCA and FLA levels in patients with CD and UC. We then compared the delta values of ASCA and FLA in CD and UC. There was a significantly greater (p = .022) decrease in ASCA levels in patients with CD than in those with UC (Figure 11). In contrast, there was no significant difference (p = .223) in the change in FLA between patients with CD or UC (Figure 12).
Figure 11: Overall Change in ASCA Levels in CD and UC. This graph depicts the change in ASCA levels on the y-axis (OD, optical density) and disease on the x-axis. The decrease in ASCA, from active disease to inactive disease, in CD was significantly larger than the decrease in UC. (\( p < .05 \))
Figure 12: Overall Change in FLA Levels in CD and UC. This graph depicts the change in FLA levels on the y-axis (OD, optical density) and disease on the x-axis. The decrease in FLA, from active disease to inactive disease, did not differ depending on the diagnosis. (p > .05)
DISCUSSION

The incidence of IBD is increasing worldwide, and researchers and physicians are highly motivated to continue to improve the diagnostic and treatment of this disease. Current standards of diagnosis, including endoscopic exam, are costly and invasive, especially for pediatric patients. Biomarkers of inflammation have been studied to help evaluate IBD. Serologic biomarkers, such as CRP and ESR, reliably indicate inflammation, but are non-specific to disease state within the GI tract (Assche, 2011; Abraham & Kane, 2012). Other serologic biomarkers such as serum ANCA and serum ASCA are slightly better at differentiating between CD and UC, with ANCA being more specific to UC and ASCA being more specific to CD (Prideaux et al., 2012; Cioffi, 2015). However, there are no serologic biomarkers that have been studied to date that have been shown to reliably differentiate between CD and UC. As such, fecal biomarkers may provide a more specific picture of inflammation in the GI tract. Fecal biomarkers, such as FLA, have been shown to provide some valuable insight into the differentiation of IBD (Abraham & Kane, 2012). More research examining fecal biomarkers in patients with IBD may be able to contribute to a less invasive and costly clinical experience for patients and families.

To date, little research has been done to examine the utility of fecal ASCA in the diagnosis of IBD. Serum ASCA measurements have been studied, and existing data suggest that levels of serum ASCA are stable over time and tell little about the dynamic nature of IBD (Prideaux et al., 2012). In one study comparing serum ASCA against fecal ASCA in patients with pouch disease, researchers concluded that fecal ASCA was better
than serum ASCA in identifying CDL in a pouch, *versus* other pouch disorders (Tang *et al.*, 2012).

Our study was the first study to assess fecal ASCA as a dynamic biomarker that can reflect disease activity and differentiate between the two forms of IBD (CD and UC). Additionally, we designed this study as a prospective study with the intention of assessing the change in ASCA levels within each patient. If fecal ASCA levels are higher during a state of active disease than during a state of inactive disease within the same patient, we can draw more robust conclusions about the utility of fecal ASCA as a dynamic marker of disease. We did not rely on absolute levels, which may vary dramatically from person to person, or use a cross-sectional study design in order to reduce the variance in our results.

In this study, we demonstrated that fecal ASCA levels measured in the same patients over time can help to distinguish between active and inactive disease states, and display distinct dynamic ranges in patients with CD *vs.* those with UC. Higher ASCA levels were more likely to be observed in patients with active CD. Higher ASCA levels were observed in patients with CD regardless of whether the patient is having active symptoms or not. We did not observe the same dynamic relationships when we studied FLA levels in patients in active and inactive disease states. While FLA levels did distinguish between CD patients with active and inactive disease, there was no difference in FLA levels in patients with active CD or UC. One caveat to keep in mind is that 12 patients were included in the analyses for UC while 35 patients were analyzed with CD.
This difference in number may have some effect on these comparisons, and ideally we would have the same number of patients in each group.

Finding a fecal biomarker that demonstrates specificity for CD or UC would be a very valuable and novel tool. A tool such as this could help to decrease the need for invasive procedures to stage and assess IBD. In the case of a new diagnosis, an ASCA level above a clinically relevant threshold could expedite the diagnostic process, perhaps prompting a more expedient referral for endoscopic evaluation, thereby avoiding a delayed diagnosis of CD. In addition, in patients with an existing diagnosis of CD, physicians may only need to rely on fecal ASCA levels to assess patients’ response to therapy, since, given that in this small study, FLA provided little additional information. In summary, measuring fecal ASCA levels in patients with CD may help physicians assess disease state and a potential need to change or escalate medical.

**Future Directions**

Given our new insight into the utility of fecal ASCA, we are now looking to new questions fecal ASCA may help us answer. Many patients, especially pediatric patients, are diagnosed with indeterminate colitis initially, which can neither be defined as CD nor UC (Bousvaros et al., 2007). Looking at changes in fecal ASCA levels in these patients may predict which diagnosis is more likely. In patients with indeterminate colitis, a high ASCA level pointing to CD may help physicians tailor treatment at an earlier stage and avoid trialing drugs that have varying effects in UC and CD. Any way to save time in the
diagnostic and treatment process is less time a patient is suffering symptoms as well as
less time secondary effects such as malabsorption and growth delay have to take effect.

Additionally, evaluating ASCA levels during treatment for IBD may help
physicians track patients’ response to new treatment and sustained response to treatments.
For example, evaluating ASCA levels in both CD and UC patients undergoing treatment
with Remicade®, a biologic immunomodulator (“Official Website for REMICADE®,”
2014), could shed light on how effective this drug is in one disease over another. More
biologic drugs are being created now with indications for IBD and other inflammatory
diseases. Many of these drugs have substantial cost, require invasive administration, and
have potential significant side effects. Having a method of testing the efficacy of a drug
sooner than observation alone could potentially avoid unwanted drug reactions, wasted
time and money, and more timely resolution of symptoms.

Our findings provide a foundation upon which larger studies looking at the utility
of fecal ASCA can be based. More information on fecal ASCA, as well as other fecal
biomarkers, will continue to provide valuable insight into the differences between CD
and UC, as well as more tools with which to improve patient care and outcomes.
REFERENCES


CURRICULUM VITAE

RACHEL P. MOJDEHBAKHSH

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108 Masons Way • Newtown Square, PA • 19073

EDUCATION

Boston University, School of Medicine, Division of Graduate Medical Sciences
M.S. in Medical Sciences, May 2015

New York University
B.A. in Psychology, May 2012
Minor in Chemistry and French, Honors in Psychology

WORK EXPERIENCE

The Phelps Lab • New York, NY • June 2012 – July 2013
Research Scientist – Held dual role of lab manager and advanced researcher

• Trained 5 research assistants in employing psychophysiological research methods to study learning and emotion
• Conducted research about neural mechanisms underlying social attribution processes, using an fMRI study and a lesion patient study, and presented this data at two national conferences
• Published two manuscripts investigating the neural mechanisms of making social judgments

Hospital for Special Surgery • New York, NY • June 2010 – March 2011
Paid Intern – Maximized efforts of the research team by helping build an innovative registry at the top orthopedic hospital

• Managed data collection for the Center for Education & Research on Therapeutics/Total Joint Replacement Registries (CERT/TJRR) to create a hospital-wide patient registry informing on the efficacy of joint replacements
• Observed surgical joint replacement procedures to further understand the factors affecting their efficacy
RESEARCH EXPERIENCE

**Inflammatory Bowel Disease Center** • Boston, MA  
**June 2014 – present**
Boston Children’s Hospital, Dr. Paul A. Rufo

*Clinical Research Coordinator* – Managed multiple clinical research projects in the Inflammatory Bowel Disease (IBD) Center
- Recruited pediatric patients, discussed fecal biomarker research with patients and families
- Collected and processed clinical samples
- Performed detailed chart reviews to complete datasets, performed data analyses
- Wrote master’s thesis focused on improving the diagnostic and treatment processes of pediatric patients with IBD

**The Molecular Imaging Program** • New York, NY  
**October 2012 – July 2013**
New York University Langone Medical Center, Dr. Alexander Neumeister

*Volunteer* – Completed the preparation phase of a drug study exploring activation of emotional neural circuits in depressed patients before and after treatment
- Prepared for Diagnostic and Statistical Manual (DSM) of Mental Disorders interviews for Post-Traumatic Stress Disorder and Depression
- Began studying mood and anxiety disorders, using multiple clinical diagnostic tools such as MRI and PET

**The Phelps Lab** • New York, NY  
**March 2010 – May 2012**
New York University, Department of Psychology, Dr. Elizabeth A. Phelps

*Volunteer Research Assistant/Honors Student* – Completed six behavioral and imaging studies and earned honors in psychology by presenting an honors thesis
- Created stimuli and programmed scripts for six projects aiming to find neural mechanisms involved in making social judgments
- Scheduled and ran hundreds of participants to collect and analyze their behavioral and fMRI data
- Became certified fMRI operator at the NYU Center for Brain Imaging to independently run imaging studies

**The Schiller Lab** • New York, NY  
**May 2011 – March 2012**
Mt. Sinai School of Medicine, Dr. Daniela Schiller

*Volunteer Research Assistant* – Led an fMRI study investigating the effects of Borderline Personality Disorder (BPD) on making social judgments
- Presented previous work and data to the Schiller Lab and collaborators at lab meetings to facilitate collaboration
Trope Lab • New York, NY
New York University, Department of Psychology, Dr. Yaacov Trope
Volunteer Research Assistant – Completed a study investigating how spatial proximity affects memory
• Created and programmed experiments, and scheduled and ran subjects to collect and analyze data

VOLUNTEER EXPERIENCE

Rosie’s Place • Boston, MA
Volunteer – Prepared meals and ran food pantry for women’s homeless shelter
• Prepared three course meals from scratch and served dining room of over 100 clients
• Assisted clients in food pantry picking out groceries
• Cleaned and reset dining room after every meal

New York City Free Clinic (NYCFC) • New York, NY
Undergraduate Volunteer – Led and facilitated the operation of the NYCFC on Saturdays
• Performed various and essential administrative duties such as scheduling patients, attending to all phone calls, relaying information to clinical team, and serving as a NYCFC resource to provide healthcare to the underserved
• Interacted with and shadowed the clinical teams to understand how a clinic is run from every position

Hospital for Special Surgery • New York, NY
Academic Visitor Program – Earned the opportunity to learn from the top orthopedic surgeons in the country
• Shadowed surgeons in the OR and during clinic hours to obtain extensive first-hand experience in surgical procedures and patient care

Hospital for Special Surgery • New York, NY
Surgical Liaison – Served as a representative for the hospital and resource about hospital procedure
• Monitored surgery status and relayed information from surgeons to families to maintain the highest standard of care

PUBLICATIONS


POSTERS AND PRESENTATIONS


AWARDS

• **Founders’ Day Award** from New York University
• **Dean’s Undergraduate Research Fund (DURF)** Awarded $1000 to complete my honors thesis project
• **Dean’s List** at New York University

SKILLS

• Proficient in Microsoft Office, SPSS, EMR software
• Languages: French