Serum Albumin as a Measure of Inflammation or Malnutrition in Inflammatory Bowel Disease: A Cross Sectional Study

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Abstract

Albumin may be both a marker of malnutrition and a marker of inflammation in various disease states but there has been very little study of the precise etiology of hypoalbuminemia in patients with Crohn's disease (CD). Malnutrition, a common complication of active CD, can lead to hypoalbuminemia. Inflammation can also lead to low albumin levels, and previous literature in other inflammatory diseases has suggested that inflammation may be more likely than malnutrition to be a primary driver of hypoalbuminemia.

This study was a single centre cross sectional study of patients with Crohn's disease in St. John's, NL. The main objectives were to examine the association between serum albumin and both inflammation and malnutrition in patients with CD and to determine if serum albumin is an appropriate indicator of one or both of these processes in patients with CD. A total of 45 patients with Crohn's disease were enrolled in the study. Serum albumin was compared with the subjective global assessment (SGA) for nutritional status, the Crohn's disease activity index (CDAI), and C-Reactive Protein (CRP), a marker of inflammation.

In our study hypoalbuminemia was independently associated with both malnutrition and inflammation in patients with CD but was most profound in subjects with both malnutrition and active inflammation.

Lay Summary

Crohn's disease is an autoimmune disease that causes inflammation in the digestive tract and can cause abdominal pain, diarrhea, low appetite, and nausea. Albumin, a protein which can be measured in the blood, may be low in some people with Crohn's disease. This is thought to be related to the inflammation itself and to malnutrition. Our study set out to determine if the low albumin levels in Crohn's disease were more strongly related to either one of those factors. We found that low albumin levels were related to both active inflammation and malnutrition, but levels were lowest in those people with both factors present.

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List of Abbreviations and Symbols

AND	Academy of Nutrition and Dietetics
ASPEN	American Society of Parenteral and Enteral Nutrition
BMI	Body Mass Index
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CKD	Chronic Kidney Disease
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
CTE	Computed Tomography Enterography
ESPEN	European Society of Parenteral and Enteral Nutrition
ESR	Erythrocyte Sedimentation Rate
GI	Gastrointestinal

GLIM	Global Leadership Initiative on Malnutrition
HREB	Health Research Ethics Board
IBD	Inflammatory Bowel Disease
MNA	Mini Nutritional Assessment
MRE	Magnetic Resonance Enterography
MUST	Malnutrition Universal Screening Tool
NL	Newfoundland and Labrador
NRI	Nutrition Risk Index
NRS	Nutrition Risk Screen
SES-CD	Simple Endoscopic Score for Crohn's Disease
SGA	Subjective Global Assessment
UC	Ulcerative Colitis
VCE	Video Capsule Endoscopy

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Chapter 1: Introduction

1.1 Background

Crohn's disease (CD) is an inflammatory disease which presents most commonly with abdominal pain and diarrhea but can also present with nausea, vomiting, and low appetite. Serum albumin levels have been shown to negatively correlate with disease activity in Crohn's disease.[1–3] Both malnutrition and inflammation may contribute to the hypoalbuminemia in Crohn's disease, but it is unclear whether either malnutrition or inflammation plays a greater role in this effect. Identifying the specific etiology of hypoalbuminemia in CD may help to guide the appropriate use of albumin as a surrogate marker in inflammatory bowel disease (IBD).

1.1.1 Malnutrition

The term malnutrition can be used to refer to any imbalance in the nutritional status of an individual. While it is recognized that overnutrition and obesity carry significant health related concerns, this study examined undernutrition, which often accompanies chronic disease. Undernutrition represents a general nutritional deficiency, and - for the purpose of this thesis - the term malnutrition will be used to refer to undernutrition. Malnutrition is a common and serious concern for chronically ill and hospitalized patients. Throughout the literature, it is associated with a myriad of health complications and health care costs, including poor surgical outcomes, poor stroke recovery, worsened subjective quality of life in patients with cancer, and increased length of hospital admission.[4–6]

It is difficult to determine a clear operational definition of malnutrition, as nutritional outcomes affect multiple organ systems and can be non-specific. In 2018, at the Global Leadership Initiative on Malnutrition (GLIM) the clinical nutrition community produced a consensus statement on the screening and diagnosis of malnutrition.[7] This statement reviewed and incorporated the current guidelines from the American Society of Parenteral and Enteral Nutrition (ASPEN)[8], the European Society of Parenteral and Enteral Nutrition (ESPEN)[9], the Academy of Nutrition and Dietetics (AND),[10] as well as multiple screening and diagnostic tools that have been previously validated. The resultant diagnostic criteria were separated into phenotypic (nonvolitional weight loss, low body mass index, reduced muscle mass) and etiologic (reduced food intake/assimilation and disease burden/inflammatory conditions). Based on this consensus statement, a diagnosis of malnutrition can be made if a patient meets at least 1 phenotypic and 1 etiologic criterion.[7]

Nutrition research currently relies upon various screening and diagnostic tools. The GLIM consensus statement was drafted in an effort to produce a universal, standardized screening and diagnostic tool for academic and clinical use. Studies to confirm the validity of this tool are ongoing.[7] At present, there are a variety of validated diagnostic tools available for research and clinical use, but there is no single gold standard for the diagnosis of malnutrition.[11]

1.1.2 Subjective Global Assessment as a Diagnostic Tool

The effects of malnutrition are non-specific and tend to impact multiple organ systems. Given the subjectivity in assessment of nonspecific symptoms, there has been an ongoing effort to find an accurate, easily administered method of assessment and diagnosis. Rather than utilizing any individual indicator in isolation, studies have found that composites of biochemical,

anthropometric, and historical findings tend to have higher diagnostic accuracy. The Subjective Global Assessment (SGA) is one example of a composite diagnostic method for malnutrition. It was based on a 1982 study by Baker *et al*., that sought to identify nutritional prognostic markers for poor outcomes in surgical patients.[11] This was then adapted by Detsky *et al*., in 1987 to create a standardized, validated tool for the diagnosis of malnutrition to be used in both research and clinical practice.[12] The SGA has been found to have an inter-rater reliability of 78%, sensitivity of 83% and a specificity of 92% for the diagnosis of malnutrition.[13,14]

The components of the SGA are summarized in Table 1. There are four features that are based upon patient history. The first of these is weight loss over both a six month and two-week period. A "small" weight loss is considered to be <5% of total body weight, whereas 5-10% body weight loss is considered "potentially significant" and >10% is considered "significant." Individuals with significant weight loss over a six-month time period, with plateauing or weight gain over a two-week period, would be considered better nourished than those who lost the same amount of weight over six months and were continuing to lose weight.[12]

The SGA also incorporates dietary intake based on history. Participants are first characterized as having normal or abnormal intake. If abnormal intake is noted, it is further classified into a suboptimal solid diet, a full fluid diet, hypocaloric liquid diet, or starvation. The duration of dietary changes is recorded as well. Gastrointestinal symptoms are reviewed and incorporated into the SGA if they have persisted for more than two weeks. These include pain on eating; anorexia; vomiting; nausea; mouth pain; early satiety; constipation; and dysphagia. Symptoms must be persistent in order to be considered significant. They are further characterized into "mild" or "severe" based on how frequently they are occurring and their course (i.e. resolving

or worsening) is also considered. Functional capacity is incorporated into the SGA. This ranges from "full capacity" to "bedridden" based on patient report.[12]

The physical examination for the SGA includes muscle wasting, loss of subcutaneous fat, edema, and ascites. Muscle wasting is assessed by examination for temporal muscle definition, clavicular protrusion, shoulder squaring, scapular/rib prominence, quadriceps atrophy, and depression of the interosseous muscles. Subcutaneous fat is assessed with examination for hollowing and depression under the eyes, measurement of the subcutaneous fat over the triceps, and significant prominence of the iliac crest and ribs.[12]

These findings are combined to assign a patient into one of three classes: well nourished (SGA-A), moderately malnourished (SGA-B), or severely malnourished (SGA-C).[12] A well-nourished individual would have no decrease in food or nutrient intake, small or no weight loss, minimal or no symptoms affecting food intake, no deficit in function, and no deficit in fat or muscle mass. Alternatively, an individual would be categorized as SGA-A if they met the criteria for SGA-B or C but had recently had adequate food intake, increase in weight and an improvement in function despite chronic deficits in muscle and fat. Those who have decreased food intake, potentially significant weight loss without stabilization, mild symptoms affecting food intake, and or moderate functional deficit or recent deterioration with mild to moderate loss of muscle and/or fat would be classified as SGA-B. This is also with the caveat that an individual who meets criteria for SGA-C but has had recent improvement of oral intake with stabilization of weight and decrease in symptoms would be classified as SGA-B rather than SGA-C. Finally, to be classified as SGA-C, an individual would need a significant deficit in intake, significant weight loss (>10%) that is ongoing, significant symptoms, and severe functional deficit and physical exam findings.

Alternatively, if there was a significant deterioration in symptoms without signs on physical exam an individual could still be classified as SGA-C.[12]

In 1987, Detsky *et al.* studied the use of the SGA on 202 patients scheduled for gastrointestinal surgery in Toronto. Five clinicians were trained on the administration of the SGA by the same instructor. Each participant in the study was independently assessed by two clinicians on the same day. They found a 91% inter-rater reliability. Prospective assessment of these patients found that both albumin and the SGA were predictive in the rate of post procedural complications such as intra-abdominal sepsis.[15] The SGA has been shown throughout the literature to be correlated with anthropometric and biochemical measures of nutritional status.[11,16,17] Notably, the SGA does not include biochemical markers, and therefore can be administered without the need for laboratory studies. More specifically, it is a method of nutritional assessment that is independent of serum albumin, which reinforces its applicability in the study of albumin and nutritional status.

Other screening and diagnostic tools like the Malnutrition Universal Screening Tool (MUST), the Nutrition Risk Screen (NRS), the Nutrition Screening Initiative (NSI) and the Mini Nutritional Assessment (MNA) have also been developed.[18–22] These tools are similar to the SGA and are based upon a composite of factors like anthropometric data, biochemical markers, and features on history that have been found to be associated with nutritional outcomes. A 2014 systematic review examined the performance of the SGA compared to other screening and diagnostic tests. Studies that enrolled surgical patients found the SGA performed well in identifying patients with malnutrition.[23] Other composite tests like the MUST, the NRI, and the NRS were found to better predict length of stay and mortality, but were limited as tools to screen

for, rather than diagnose, malnutrition.[23] Two studies included in this systematic review specifically enrolled patients with gastrointestinal diseases. These studies found that, in patients with gastrointestinal disease, the SGA had a high prognostic value in predicting length of stay[24] and was more sensitive in the diagnosis of malnutrition than anthropometric measures.[19]

This systematic review concluded that the SGA is a more valid tool for nutritional diagnosis when compared to diagnostic methods such as anthropometric measures and biochemistry. The NRS was suggested as a preferred screening tool for malnutrition in general patients. For diagnostic tests, the MNA was suggested for geriatric populations. The SGA performed well in studies of gastroenterology patients and remains a reasonable diagnostic tool. This review highlights the need for a standardized, universal, and validated assessment method for malnutrition.[23] The GLIM criteria were proposed in an effort to fill this gap, but these have yet to be validated and so researchers and clinicians are left with multiple assessment options for malnutrition, including the SGA.[7]

Table 1. A Summary of the SGA

Adapted from Detsky *et al.*, 1987

Nutritional Intake - Assessed over 6 month period and 2 week period		
1) Adequate 2) Suboptimal solid food diet 3) Full fluids/Nutritional supplements 4) Clear fluids/starvation		
Weight - Changes over 6 month period and 2 week period		
1) None/Mild (<5% loss) 2) Moderate (5-10% loss) 3) Severe (>10% loss)		
Symptoms that affect oral intake (ie abdominal pain, dysphagia, early satiety)		
Functional Capacity - Over 6 month period and 2 week period		
1) No dysfunction 2) Difficulty with ambulation/daily tasks 3)Difficulty with daily tasks 4)Bedridden		
Physical Exam		
Loss of body fat	Loss of muscle mass	Presence of edema
Overall SGA Rating		
A - Well nourished, no decrease in food intake, <5% weight loss, no/minimal symptoms, no functional deficits, no physical exam finding	**B -** Mild/mod decrease in intake, 5-10% weight loss without stabilization or gain, mild/moderate symptoms, moderate functional deficit, mild/moderate physical exam findings	**C -** Severe deficit in food intake, >10% weight loss, significant symptoms, severe functional deficit, severe physical exam findings

1.1.3 Albumin Physiology

Biochemical nutritional markers might theoretically offer a simple, objective method of identifying malnutrition. Historically the most commonly used biochemical marker for nutritional status has been serum albumin, which is a protein that is synthesized from amino acids in the liver and accounts for approximately 60% of an adult's blood plasma protein. Normal values for serum albumin range from 35 g/L to 50g/L. The synthesis of albumin occurs in the hepatocytes, and its regulation relies heavily upon the colloidal oncotic pressure near the site of synthesis. In early states of fasting, the body is deprived of dietary amino acids and intrahepatic proteins are broken

down and used to continue production of albumin. This is in contrast to other hepatic proteins, which tend to obtain amino acids from the catabolism of muscle protein in fasting states. This mechanism allows for a more rapid reversibility in albumin homeostasis, as this protein can be synthesized in the liver in 15-30 minutes. Longer term nutritional deficits lead to a decrease in albumin mRNA, and albumin deficits which are less easily reversed.[25]

Once albumin is released from the liver, it is distributed throughout the intravascular (30-40%) and extravascular spaces (60-70%). Given this, excess fluid in the extravascular space seen in volume overload states like heart failure or renal failure can lead to a low measured serum albumin. After hepatic release, the half-life of albumin is 17-19 days before catabolism occurs.[25] Albumin production may be decreased for multiple reasons, including a lack of "building block" amino acids and enzymes available through the diet. Inflammatory disease states can also lead to a decrease in albumin, as the liver utilizes those amino acid "building blocks" selectively to create inflammatory proteins such as c-reactive protein, serum amyloid A, and alpha-1-antitrypsin.[18,26] These proteins function to improve host defenses and immune response by mobilizing leukocytes and increasing blood flow.

1.1.4 Albumin in Malnutrition

The liver uses amino acids as the building blocks for albumin synthesis. When a patient has low protein intake, amino acids are not readily available for this synthesis and are mobilized first from intrahepatic proteins to quickly reverse the deficit in albumin. In states of true and prolonged fasting, insulin secretion will be decreased, which results in the mobilization of fatty acids, and the breakdown of skeletal muscle in order to maintain visceral protein synthesis.[27] Through these mechanisms, albumin synthesis can theoretically be decreased in the setting of

decreased nutritional protein intake. Due to its nature as a negative acute phase reactant - or a biochemical marker that negatively correlates with inflammation - any inflammatory state or systemic illness could also lead to a decrease in albumin synthesis.[25] This potentially confounds the relationship between malnutrition and serum albumin. In order to examine this association, researchers have studied disorders such as anorexia nervosa, which is characterized by severe caloric restriction, but is typically seen in otherwise healthy individuals without systemic illness or inflammation.

A 2015 systematic review of studies of non-diseased individuals with caloric restrictions found that serum albumin remained normal until the extremes of starvation (Body Mass Index (BMI) <15 or >6 weeks of severely decreased intake).[28] This suggests that in otherwise healthy individuals, serum albumin may be an unreliable marker of under-nutrition until it has reached very extreme states. Although albumin has been viewed primarily as a nutritional marker due to its physiology, in mild, early cases of nutritional deficiency in patients without comorbidities, this may not be accurate.[18] In patients with comorbidities such as inflammatory conditions, the baseline synthesis and distribution of albumin may be affected. With this baseline deficiency in albumin synthesis, it is possible that mild to moderate malnutrition may lead to a sustained decrease in serum albumin levels that, in otherwise healthy individuals, would be seen only in severe undernutrition.

There is an understandable interest in the use of serum albumin as a marker of malnutrition. When compared to the SGA, measuring serum albumin is less subjective and less time consuming for the patient and health care provider. As a simple lab test, it would be a relatively easy,

inexpensive, and expedient surrogate measure for nutritional status. At present, however, albumin has been understudied as a specific diagnostic measure of malnutrition in chronically ill patients.

1.1.5 Malnutrition in Crohn's Disease

Patients with inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) have a higher risk for malnutrition than the general population.[29–31] In Crohn's disease, any part of the gastrointestinal (GI) tract from the mouth to the rectum can be affected by inflammation, though it is seen most commonly in the small bowel. Depending on the exact location of disease activity, patients with CD often experience significant abdominal pain, decreased appetite, and diarrhea. In contrast, the inflammation in ulcerative colitis is limited to the colon and typically presents with frequent bloody diarrhea.[27,32,33]

Malnutrition in IBD occurs by two primary mechanisms. The first of these is through lack of absorption in the GI tract. This can occur if an individual has undergone surgical resection of a portion of their bowel as a result of their inflammatory bowel disease. It can also occur due to active inflammation in the GI tract. Gut mucosa becomes inflamed in IBD, which leads to a decreased transit time through the small and large bowel and to decreased absorption of nutrients due to inflammation of the mucosa itself. Because Crohn's disease most frequently affects the small bowel, and the small bowel is the region of the GI tract that is responsible for the majority of nutrient absorption, this mechanism of malnutrition is more commonly seen in CD than in UC.[27,29–31]

Both CD and UC can lead to decreased oral intake for a variety of reasons. Individuals who experience frequent, loose bowel movements may reduce their oral intake in an effort to reduce

their stool output. Decreased appetite associated with active disease may also lead to decreased oral intake. In many cases abdominal pain is exacerbated by eating, which also motivates patients to decrease their intake in an attempt to decrease pain. This mechanism of malnutrition is seen in both forms of IBD.[27]

Because of their high risk for malnutrition, determining if IBD patients need nutritional support through dietary supplementation is an important aspect of their medical care. Patients with IBD who are malnourished have a diminished quality of life, more difficulty with wound healing (which is particularly important as many of these patients are on immunosuppressive medications), and worse outcomes if surgery is required.[5] If a simple, standardized tool for the diagnosis of malnutrition were available, interventions such as nutritional supplementation could be implemented and allied health members could be involved in care earlier with a more easily discernible end point.

1.1.6 Albumin in Crohn's Disease

In patients with IBD, hypoalbuminemia is influenced by many factors in addition to nutritional status. One cause of hypoalbuminemia in these patients is related to albumin's role as a negative acute phase reactant in any inflammatory condition. As reviewed above, active inflammation that is seen in diseases such as Crohn's disease is associated with the production of inflammatory proteins. With increased synthesis of inflammatory proteins, the synthesis of albumin is decreased and hypoalbuminemia occurs.[18]

Some patients with IBD may also develop protein losing enteropathy. Increased permeability of the inflamed mucosa leads to luminal loss of protein through the GI tract. In these

patients, high amounts of protein are excreted through the gastrointestinal tract, which leads to low levels of albumin remaining in the body.[27]

Serum albumin may also be decreased in patients with IBD due to malnutrition. The degree of malnutrition in these patients varies significantly.[25,34] With severe and prolonged disease, some patients may develop extreme malnutrition. The majority of patients followed in IBD clinics often have less severe symptoms and milder, if any, malnutrition.[35] Based on studies of albumin in caloric restriction, it has been theorized that malnutrition alone should not be the primary driver of hypoalbuminemia in patients without an underlying disease until they are severely malnourished.[28] At this point in time, it is unclear whether serum albumin correlates more closely with the level of nutrition or the activity of the disease in patients with IBD.

It has been shown that serum albumin has a negative correlation with disease activity in IBD patients.[1–3] An improved understanding of this association would be beneficial in the monitoring and management of these patients, who may require dietary interventions or treatment optimization. Our study aims to investigate the use of serum albumin as a surrogate marker of inflammation and malnutrition in patients with Crohn's Disease.

1.1.7 Measurement of Crohn's Disease Activity

The primary characteristic of Crohn's disease is inflammation. As reviewed above, one of the principal ways in which Crohn's disease is distinct from ulcerative colitis is in the location of inflammation.[27] While inflammation can be present in the colon in both ulcerative colitis and Crohn's disease, the latter can also affect the entirety of the GI

tract, with predominance in the small bowel. Measurement of gastrointestinal inflammation is a key component of disease and treatment monitoring, and there are various biochemical, imaging, and scoring methods to determine inflammatory disease activity.[36] Currently, the recommended treatment goal is to target therapies to achieve mucosal healing. In order to determine if this goal has been met, the mucosa must be either directly visualized or a surrogate marker for mucosal inflammation must be measured.[36,37]

Methods of determining the degree of inflammation in Crohn's disease:

1) Endoscopy - Direct visualization of the mucosa and biopsy is typically considered the gold standard for measurement of disease activity in Crohn's disease. This can be done via colonoscopy - a procedure which allows the endoscopist to directly view the mucosa and any inflammation that may be present in certain parts of the gastrointestinal tract.[38] As CD most commonly affects the colon and terminal ileum (the end of the small bowel), this method of disease activity monitoring is helpful in many CD patients. Endoscopists can grade the severity of Crohn's disease activity using the simple endoscopic score for Crohn's disease (SES-CD) in order to objectively describe disease activity.[39]

   Colonoscopies can routinely visualize the full colon and the distal ileum.[38] The other segments of the small bowel cannot be visualized at colonoscopy. Similarly, upper endoscopies can visualize the esophagus, stomach, and duodenum, but routine upper endoscopies cannot be relied upon to determine disease activity beyond the duodenum.[40] Push or balloon enteroscopies can be used to increase the extent of the examination to include the entire duodenum, the jejunum, and much of the ileum. Enteroscopy carries a higher risk of complications than upper endoscopy due to the prolonged time of examination and sedation,

and increased length of bowel examined. It also carries increased technical challenges with a higher likelihood of an incomplete examination and, in the case of balloon enteroscopy, may require a therapeutic endoscopist with advanced training in the modality.[40] Routine direct visualization of the mucosa is therefore limited in the assessment of disease activity for patients who have inflammation in the jejunum and much of the ileum.[40] Figure 1 illustrates the locations within the GI tract that are accessible for endoscopic visualization as compared to the common or possible locations of inflammation in Crohn's disease.

Endoscopic assessment is relatively invasive for the patient and time consuming for both the patient and clinician. The patient must undergo a purgative bowel preparation in order to fully clear out the bowel to allow for appropriate visualization. This typically entails a large volume preparation of four litres of a polyethylene glycol-based preparation or a smaller volume preparation like sodium picosulfate/magnesium citrate, which would need to be taken with plenty of water. These bowel preparations act as osmotic laxatives and draw water through the colon. From the patient's perspective, this results in significant watery diarrhea to clean out the bowel. From the endoscopist's perspective, this allows for adequate visualization of the mucosa throughout the bowel in order to allow the endoscope to advance safely with good visibility and to ensure that any abnormalities are seen.[41] Patients typically undergo sedation in order to make the procedure more comfortable,[42] which means losing at least one full day of work for the procedure. Colonoscopy also carries the risk of bowel perforation (a hole or tear in the lining of the bowel wall), bleeding, and sedation complications.[43] For the health-care system, colonoscopy requires a skilled endoscopist, nursing staff trained in endoscopy, and (depending on method of sedation) an

anesthesiologist. Despite these factors, colonoscopy remains an excellent method of objectively assessing disease activity, but it is time and resource intensive and is associated with more risk than some other methods.[43]

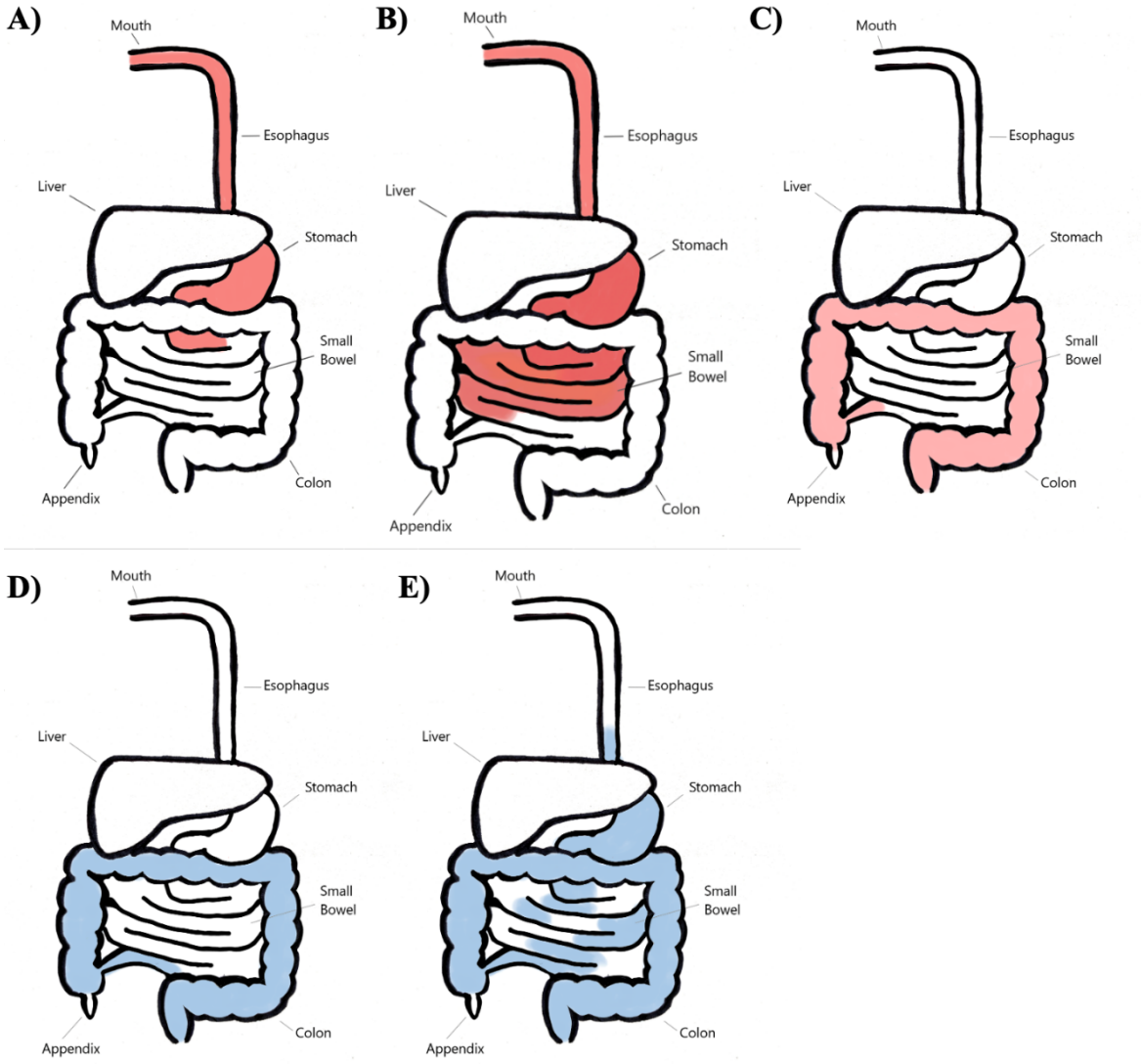

Figure 1. areas of endoscopic visibility vs. Crohn's disease activity

Original figure by author. (A) Areas of the GI tract that can be directly visualized with Esophagogastroduodenoscopy; (B) Areas of the GI tract that can be directly visualized with enteroscopy; (C) Areas of the GI tract that can be directly visualized with colonoscopy; (D) Most common areas of inflammation in Crohn's disease; (E) Most common and other possible locations of inflammation in Crohn's disease - typically seen in a patchy distribution

2) Enterography - Magnetic resonance enterography (MRE) and computed tomography enterography (CTE) are medical imaging techniques which can be used to assess inflammation in Crohn's disease in areas that are inaccessible via endoscopy. In patients who have small bowel inflammation, MRE and CTE can be used as a diagnostic test and a monitoring tool. Both of these techniques use an oral drink of approximately one liter to provide contrast between the bowel lumen and the surrounding tissue.[44] Given that the most common locations for Crohn's disease activity are the colon and terminal ileum, which are both accessible by endoscopy, the yield of enterography in CD is highest in the subset of patients who have small bowel inflammation proximal to the terminal ileum.[40,44]

3) Capsule Endoscopy - Video capsule endoscopy (VCE) is another method of directly visualizing the mucosa in Crohn's disease. This test involves the patient swallowing a camera (video capsule) that is the size of a large pill. Images are taken repeatedly as the capsule transits through the bowel and transmitted wirelessly to a pack that is strapped to the patient's torso. These images are then reviewed to look for inflammation throughout the GI tract. Video capsule endoscopy requires a gastroenterologist trained in interpretation of the images, as well as the equipment necessary for the test. It is relatively low risk, with a 1% chance of capsule retention, which could necessitate surgical removal in severe cases.[45] This is, unfortunately, a slightly higher risk in patients with small bowel Crohn's disease, as Crohn's disease can lead to narrowings or strictures throughout the GI tract that can result in obstruction when a video capsule is trapped in a narrow stricture.[46,47]

VCE in Crohn's disease patients can be used to determine the extent and severity of inflammation throughout the small bowel and, like enterography, may be of particular

importance in visualizing disease in areas not accessible by endoscopy. It is, however, more invasive, resource intensive, and higher risk than measurement of biochemical markers in routine monitoring.[47]

4) Crohn's Disease Activity Index (CDAI) - The CDAI was developed in 1976 as a method of assessing the overall activity of CD. It is a validated, weighted score of a variety of factors that make up active disease. It assesses disease activity by recording symptoms and signs over a one-week period. These include: number of bowel movements, severity and frequency of abdominal pain, associated extraintestinal manifestations of CD, use of agents to slow bowels (i.e. loperamide), hematocrit, weight (from baseline), presence of abdominal mass on exam, and the patient's subjective rating of their general well-being.[48] The CDAI used in this study can be found in appendix 2.

   A CDAI score of <150 represents disease remission, while a score of ≥ 150 represents active disease. The CDAI is a non-invasive, holistic method of reviewing disease activity in patients with Crohn's disease.[48]

5) Serum inflammatory markers - Serum inflammatory markers are often used to estimate disease activity in patients with CD. As it is an inflammatory disease, biochemical markers that measure inflammation are typically elevated in states of active disease. C-Reactive Protein (CRP) is a protein produced in the liver that increases acutely in the presence of inflammation. As a serum marker, it is relatively non-invasive and inexpensive to obtain. It is a less expensive and more rapidly responsive marker than erythrocyte sedimentation rate (ESR), which is a similar inflammatory marker that had been the previous standard serum marker for inflammation.[49]

As a measure of inflammation, CRP can be relatively non-specific. It increases in response to inflammation in general and can be elevated in patients with a wide variety of inflammatory diseases as well as burns and infections. It is used to monitor Crohn's disease activity as a marker of inflammation in patients with other signs or symptoms of the disease. As a non-specific marker, it must be considered in the appropriate clinical context as CRP elevation may not always represent inflammation specific to Crohn's Disease activity.[48]

6) Fecal calprotectin - Calprotectin is a protein found in neutrophils, a particular type of white blood cell. In the absence of inflammation in the bowel, there is a minimal amount of calprotectin secreted by the gastrointestinal tract into the stool. In cases of mucosal inflammation or cell death in the gastrointestinal tract, an increased amount of calprotectin is released into the stool. This can then be measured in a stool sample. Elevated fecal calprotectin is a more specific measure of inflammation than CRP in patients with CD.[50]

Fecal calprotectin is most sensitive in measuring inflammation in the colon and the distal small bowel. Inflammation higher up in the GI tract may be present even with a normal fecal calprotectin. As a stool test, it remains a non-invasive method of measuring inflammation in the gastrointestinal tract. While it is non-invasive, there can be difficulty with patient compliance with take-home stool kits, and obtaining results can be less reliable when ordering a fecal calprotectin than when ordering serum markers.[50,51]

1.2 Research Question

In order to investigate the association between serum albumin and disease activity in individuals with Crohn's disease, we proposed the following research question:

1. In Crohn's disease, what is the association between serum albumin and two factors – (1) inflammation and (2) malnutrition, and;

2. Is serum albumin an appropriate diagnostic measure of one or both of these factors in patients with CD.

For the purposes of investigating this question, CRP was used as a marker of inflammation, the SGA was used as a diagnostic score for malnutrition, and the CDAI was used as a measure of overall Crohn's disease activity.

1.3 Conceptual Framework, Theory, and Hypothesis

The conceptual framework used for this research involves weighted etiologies.[52–54] As illustrated in Appendix 3 and discussed above, there are various potential etiologies for hypoalbuminemia in IBD, and each may have a different level of causal significance for a low serum level. Therefore, correcting one factor which may be playing a small role in causing hypoalbuminemia will not lead to a significant change in albumin. Each of the factors (i.e., malnutrition, inflammation, protein loss) is caused by different pathophysiologic processes in IBD. Malnutrition is caused by various mechanisms in Crohn's disease such as decreased food intake and poor nutrient absorption. Inflammation on its own leads to hypoalbuminemia by decreasing the synthesis of albumin in the liver in order to increase the synthesis of acute phase reactants and by leading to increased protein loss from the gut.[27]

Based on studies of non-diseased individuals with undernutrition, it is possible that albumin will not decrease due to mild-moderate malnutrition alone.[28] Considering the physiology of albumin synthesis and the patterns observed in non-diseased individuals and those with other

disease states, inflammatory processes in Crohn's disease may contribute more significantly to hypoalbuminemia than undernutrition does. However, it is also possible that an underlying deficiency in albumin synthesis due to inflammation may lead to an increased tendency toward hypoalbuminemia when any degree of malnutrition is introduced.

This study tests the following null hypotheses: (1) Low serum albumin is not correlated with the SGA and therefore cannot be used in the assessment of malnutrition in individuals with CD, and (2) Low serum albumin is not correlated with CRP and therefore cannot be used in the assessment of inflammation in individuals with CD. Therefore, if the correlations are statistically significant, the null hypotheses will be rejected to determine that (1) low serum albumin is a diagnostic indicator of malnutrition, and/or (2) low serum albumin is a diagnostic indicator of inflammation.

Chapter 2: Literature Review

2.1 Albumin and CD Activity - a brief overview

A clear correlation between IBD activity and serum albumin concentration has been demonstrated in the literature;[1–3] however, the topic has been understudied in recent years. For instance, Seo *et al*. found that serum albumin had a strong association with IBD - as disease severity increased symptomatically and endoscopically, serum albumin concentration decreased.[3] Similarly, Powell-Tuck found that low serum albumin concentrations correlated with decreased nutritional intake as well as increased intestinal inflammation in IBD patients.[1] These studies did not indicate whether the correlation with disease severity was related to the inflammation associated with the disease, or with the malnutrition that may be seen with increasingly severe IBD.

2.2 Albumin as a marker of malnutrition vs. inflammation in CD

To investigate this relationship, a search of the literature was completed, including MEDLINE, PubMed, CINAHL, and the Cochrane Database. The bibliographies of the relevant studies, review articles, and guidelines were also reviewed.

The key-terms “albumin” AND “malnutrition” OR “nutritional status” AND “Inflammatory bowel disease” OR “IBD” OR “Crohn’s disease” OR “ulcerative colitis” AND “SGA” OR “Subjective global assessment” were used. Notably, there is a relative paucity of literature in patients with IBD specifically examining the use of albumin as a primary diagnostic indicator of inflammation relative to malnutrition. After examining the 14 non duplicate articles

that this search yielded, only two articles explicitly related to the topic were found, indicating a gap in the literature.

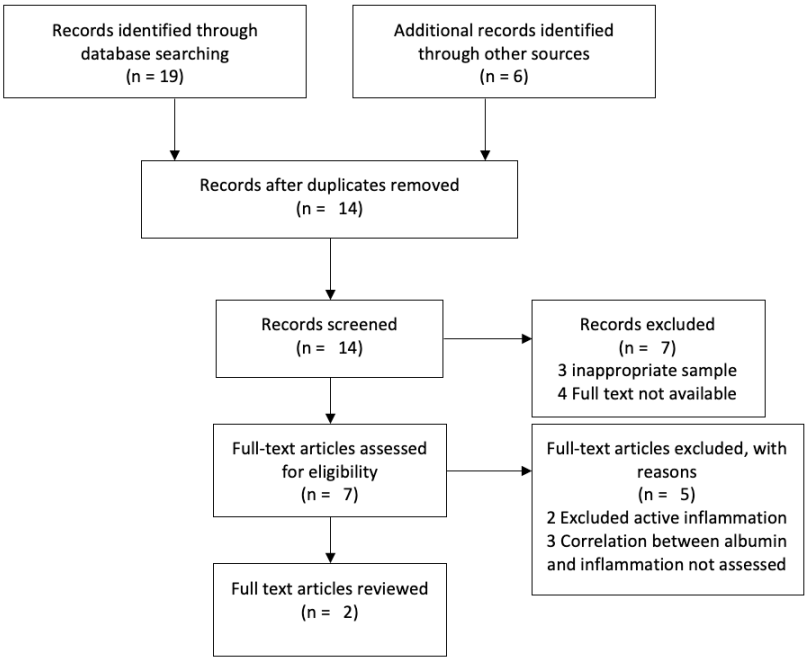

Figure 2. Literature review flow diagram

Flow diagram of the literature search for articles on inflammation, nutritional status, and albumin in inflammatory bowel disease. Search method outlined in the body of the text

In 1993, Novacek *et al.* investigated the association of serum pseudocholinesterase (PCHE) and albumin with malnutrition and inflammation in patients with CD.[55] Albumin and PCHE values were compared in well-nourished and malnourished patients with both active and quiescent CD. Both proteins were found to be generally lower in malnourished than well-nourished patients. In patients with active Crohn's disease by CDAI there was a statistically significant difference in serum albumin between those who were malnourished and those who were well nourished with lower values in those who were malnourished. However, this difference was not seen in patients

with quiescent CD. Furthermore, serum albumin was lower in patients with active CD than those with quiescent CD. These results support the hypothesis that the mechanism for hypoalbuminemia is multifactorial in CD and that hypoalbuminemia is not seen in patients with malnutrition in the absence of inflammation. The definition of malnutrition as less than 90% ideal body in this study is suboptimal compared to the SGA.[55]

Cabral *et al*. found that albumin was much more strongly correlated with Crohn's disease activity (sensitivity 100%) than with malnutrition. To investigate this, the researchers compared serum albumin with (1) inflammation using erythrocyte sedimentation rate (ESR), (2) malnutrition using anthropometric measurements, and (3) disease activity using the Harvey Bradshaw index. Overall, there was a high rate of both malnutrition and hypoalbuminemia in the patients with IBD included in their study. The severity of malnutrition was not found to be correlated with the degree of disease activity or serum albumin level. There was a strongly significant correlation between disease activity and serum albumin levels, as well as a statistically significant relationship between serum albumin and inflammation as measured by ESR - though the strength of this correlation was not quantified.[56]

No other studies examining the association between serum albumin, malnutrition and inflammation in CD were found. While this association is understudied in IBD, there is research examining this association in other systemic inflammatory disorders, such as chronic kidney disease (CKD).

A similar search was conducted in the same databases with "chronic kidney disease" rather than "inflammatory bowel disease" as a key term. It yielded four relevant studies. A 2012 study by Gama-Axelsson *et al*., examined the relationship of serum albumin to inflammation and

malnutrition, using CRP and the SGA, respectively, as surrogate measures. They split patients into two groups – incident and prevalent – who were just beginning dialysis or who had been on dialysis for >6 months. They used multiple linear regression to analyze multiple factors contributing to hypoalbumemia. Overall, they found that albumin was more strongly correlated with medical comorbidities and inflammation, though there was also a statistically significant correlation between albumin and SGA status.[34]

In a cross-sectional study done in Morroco in 2017, Essadik *et al*. concluded that there was no difference in the correlation between serum albumin and various measures of malnutrition and inflammation, including the SGA and CRP. While serum albumin was very strongly correlated with nutritional status (by SGA), it was also very strongly correlated with inflammation by CRP. Overall, they concluded that serum albumin had very poor specificity (26.3%) for malnutrition in this patient population.[57]

Ruperto (2016) similarly found that albumin alone was not an appropriate diagnostic measure of malnutrition. This study examined 80 hemodialysis patients who were split into well-nourished and malnourished groups. The researchers evaluated the use of serum albumin, mid arm muscle circumference, and standardized body weight to diagnose malnutrition and found that the three together were useful in the diagnosis of malnutrition, but that albumin alone had the weakest correlation with malnutrition. No investigation was done in this study into albumin's relationship with inflammation.[58]

Finally, in a study of 128 patients on hemodialysis, Qureshi split patients into well-nourished, mildly malnourished, and severely malnourished groups based on the SGA. Qureshi

found that serum albumin was a strong predictor of nutritional status. However, they also noted that this was heavily influenced by age, comorbidities, and CRP.[59]

In summary, there has been very little research done into the use of serum albumin in the assessment of inflammation or malnutrition in IBD. This underscores the gap in the literature specifically examining this association. The studies that have been published appear to demonstrate that serum albumin is a stronger indicator of inflammation than of malnutrition in IBD. This seems to correlate with much of the research into serum albumin and its role in inflammation and malnutrition in CKD.[34,57–59]

CKD is similar to IBD in that it leads to high levels of inflammation, but the mechanism by which hypoalbuminemia occurs in each disease may be different. IBD can result in protein wasting from the gut, whereas CKD patients lose protein via the kidneys. Furthermore, patients with IBD commonly have fewer comorbidities and represent a younger cohort than those with CKD. These differences may influence the relationship between albumin, undernutrition and inflammation in patients with IBD which invites further study of this topic.

Chapter 3: Methods

3.1 Ethics Statement

This study was approved by the Health Research Ethics Board of Newfoundland and Labrador (HREB # 2019.058). It was also approved by the regional health authority, Eastern Health, through the Research Proposal Approval Committee.

Participants included in this study provided written informed consent prior to participation in the study.

3.2 Participants

The study population was made up of patients with Crohn's disease who were followed by gastroenterologists at the Health Sciences Centre gastroenterology clinics in St. John's, NL, Canada. Patients were asked to participate at their regular follow up appointments.

3.2.1 Inclusion Criteria

To be included in the study, patients had to meet the following criteria:

1. Have a diagnosis of Crohn's disease;
2. Be followed by the GI clinics at HSC and receive regular blood work and;
3. Be over the age of 18.

3.2.2 Exclusion Criteria

Patients were excluded from the study if they:

1. Opted not to participate
2. Had a history of mental health concerns that could have negative associations with questions regarding weight or health such as:
    a. Illness anxiety disorders;
    b. Eating disorders (anorexia or bulimia)
3. Had other inflammatory or protein losing diseases that would contribute to low albumin:
    a. End stage renal disease, including hemodialysis patients;
    b. Nephrotic syndrome;
    c. Liver Cirrhosis

3.2.3 Sample Size Calculation

$$N = [(Z_\alpha + Z_\beta)/C]^2 + 3$$

To determine the sample size necessary for this study, two calculations were carried out using the above formula described by Hulley et al.[60] The rationale for the use of two calculations is that two separate associations were being studied (i.e. malnutrition and inflammation). The

larger of the two calculations was implemented as the study's minimum required sample size. The expected correlation coefficient (r) was found using previously described correlations found in studies on end stage renal disease, as there were no previous values in the IBD literature. The type I error rate was set at 0.05 and the type II error rate at 0.20 (power = 80%). The standard normal deviate for Z_α set at 0.05 is 1.96, and the standard normal deviate for Z_β set at 0.20 is 0.8416.

The first calculation used an expected correlation coefficient, between albumin and inflammation (CRP), of 0.37 as demonstrated by Gama-Axelsson et al. in 2012.[34] This resulted in a minimum sample size of 55 patients for a power of 80% and confidence interval (CI) of 95%.[34]

$$N = \left(\frac{(1.96 + 0.8416)}{0.3884}\right)^2 + 3 = \left(\frac{2.8016}{0.3884}\right)^2 + 3 = 52 + 3 = 55$$

The second calculation used an expected correlation coefficient of 0.284 between serum albumin and the SGA based on a 2003 study by Fein et al.[61] With a power of 80% and a CI of 95%, this resulted in a minimum sample size of 95 patients, which is the larger of the two calculations and was the intended sample size used for this study.

$$N = \left(\frac{(1.96 + 0.8416)}{0.2920}\right)^2 + 3 = \left(\frac{2.8016}{0.2920}\right)^2 + 3 = 92 + 3 = 55$$

3.3 Research Setting

In Newfoundland and Labrador in 2012, there were 2008 patients with Crohn's disease.[35] Due to the distribution of health care throughout Newfoundland and Labrador and the population

density throughout the province, the majority of patients requiring specialist gastroenterology are seen in St. John's, NL at either the Health Sciences Centre clinics or at a community clinic associated with St. Clare's Mercy Hospital. The clinics at the Health Sciences Centre employ six of the city's nine gastroenterologists. Due to these factors, it was felt that the highest yield in recruiting patients with Crohn's Disease to the study would be at the Health Sciences Centre Gastroenterology Clinics.

3.4 Data Collection Procedure

At the conclusion of a patient's regular follow up GI appointment, the gastroenterologist involved in their care informed them of the option to participate in the study. If a participant was interested, the principal investigator was informed and met with the patient at the end of the appointment. After informed consent was obtained and any remaining questions answered, the questionnaire components of the SGA and CDAI were first administered and the participants medical history was clarified. The physical exam components were then administered. The physical exam components were completed together following the questionnaires in order to expedite the process to minimize the disruption to clinic flow and the participants day. After this 10-15 minute assessment, the participant had no further follow up requirements.

Computerized charts and endoscopy notes were reviewed for basic demographic information, location of disease, comorbidities, and biochemical tests. This data was collected after the SGA and CDAI were documented, in order to minimize the potential for these subjective questionnaires to be biased by knowledge of the participants' albumin or inflammatory markers.

3.5 Instrumentation

Patient assessments with the SGA and CDAI were performed prior to the chart review in order to prevent any unintentional bias in the subjective portion of these tests. They were collected by the primary investigator in order to ensure no inter-observer variability.

3.5.1 Subjective Global Assessment

As reviewed above, the subjective global assessment (SGA) was selected as a validated means of assessing malnutrition in patients with gastrointestinal disease. The SGA was administered to each participant by the principal investigator. This was done using the questionnaire in Appendix 1. The physical examination component of the SGA includes examination for loss of subcutaneous fat (under the eyes, triceps, and ribs), loss of muscle (temporal, clavicular, scapula/ribs, shoulder squaring, quadriceps, interosseous muscles), and presence of edema. Examination was performed by the principal investigator, who has received training in administration of the SGA throughout medical education in didactic sessions and by working with dietitians clinically.

3.5.2 Crohn's Disease Activity Index

The Crohn's Disease Activity Index was administered to each patient involved in the study. The questionnaire is attached in Appendix 2. The questions were read out by the investigator and definitions were explained if the participant required clarification. The physical exam component of the CDAI requires only an abdominal exam to identify any potential abdominal masses. This exam was performed by the principal investigator, who has received training in physical examination techniques.

3.6 Data Collection

Electronic health records were reviewed in order to obtain biochemical markers, patient demographics, and comorbidities necessary for the study. Electronic health records were reviewed the day after the SGA and CDAI were collected. If a participant had undergone their routine blood work within 1 month of the SGA and CDAI being collected, these values were used. If a participant did not have blood work documented on initial review, records were again reviewed one month following the initial meeting. If they did not have blood work collected by that point in time, they were not included in the study.

3.6.1 Biochemical Markers

Serum Albumin (normal range 35 g/L to 50 g/L) - Serum albumin is frequently measured on routine blood work in patients with IBD. Serum albumin was recorded if it was collected within one month of the participants' SGA and CDAI. Blood samples had been collected as per Eastern Health lab protocol with standard venipuncture technique. Serum albumin was measured as an albumin BCP assay for the quantification of albumin in human serum using an Abbott Architect c-series clinical chemistry analyzer.[62]

*C-Reactive Protein (*normal range 0 mg/dL to 8mg/dL) - CRP is also routinely measured in follow up of IBD as a measure of inflammation. Participants' CRP was recorded if it was collected within one month of their SGA and CDAI. Blood samples had been collected as per Eastern Health lab protocol with standard venipuncture technique. CRP was measured as a high sensitivity CRP with the multigent CRP vario – a latex immunoassay – using an Abbott Architect c-series clinical chemistry analyzer.[63]

Hematocrit (normal 47% for males, 42% for females) - Hematocrit is an important value in the calculation of a patient's Crohn's Disease Activity Index. This was collected from the electronic health record for use in the CDAI. Blood samples had been collected as per Eastern Health lab protocol with standard venipuncture technique. Hematocrit was measured using a Beckman Coulter Unicel DxH 800 hematology analyzer.[64]

3.6.2 Demographics and Comorbidities

On assessment, participants age, biological sex, comorbidities, previous surgeries, and disease location were reviewed with them. This information was also collected using the electronic health record by reviewing their most recent endoscopy records and clinic notes from their most recent gastroenterology appointments.

3.7 Data Collection Extenuating Circumstances

As reviewed in the attached COVID-19 Impact Statement, data collection was limited due to the effects of a global pandemic. By March of 2020, 45 participants had been included in the study. On March 18, 2020 a Public Health State of Emergency was declared in Newfoundland and Labrador as a response to COVID-19. In light of this, the Health Research Ethics Board and Memorial University independently mandated the cessation of any in-person data collection for an indefinite period. With these restrictions in place, the current data was reviewed and discussed with the supervisory committee for this project as well as the Assistant Dean of Research and Graduate Studies. Due to these unprecedented circumstances, it was decided that proceeding with

a limited sample size was acceptable and data collection was stopped at 45 participants rather than the target sample size of 95.

3.8 Statistical Analysis

Univariate analysis of the associations between serum albumin, serum CRP concentrations, CDAI, and SGA were determined using Chi-square tests, Pearson correlation coefficients for continuous data and Spearman rank correlation coefficients for categorical data. Multiple linear regression was used for multivariate analysis. Because of the skewed distribution of serum CRP concentrations and the CDAI scores, the log-transformed CRP concentration and CDAI were used for correlation comparisons. A two-way ANOVA was run to examine the effects of inflammation (CRP) and malnutrition (SGA) on serum albumin. Measurable continuous parameters were reported as means ± standard deviation (SD) and groups were compared using Student t-tests. A p-value <0.05 was regarded as statistically significant.

Data were analyzed using IBM's SPSS Statistics (version 26) and SYSTAT 13 for Windows (Systat Software, Inc., San Jose, CA, USA)

Chapter 4: Results

4.1 Enrollment Process

A total of 48 participants were approached to take part in the study between September 16, 2019 and March 13, 2020. Of these, two patients were excluded based on exclusion criteria, and one was excluded due to a lack of available data in the electronic medical record. 46 participants were initially assessed in the GI clinics. One participant had no serum albumin level in the electronic medical record, so their data was excluded from the study.

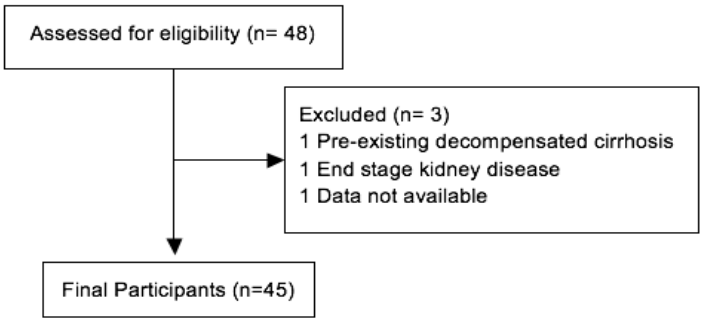

Figure 3. Flowchart of enrollment process

4.2 Patient Characteristics

Participants varied in age from 19 to 79 with an average age of 51.5 years. There were 22 male participants (49%) and 23 female (51%). Overall, 18 participants had active disease (40%), while 27 had disease in remission (60%). In terms of nutritional status, 30 participants had normal nutrition (67%) and 15 participants (33%) were found to have mild-moderate malnutrition. No participants were found to be severely malnourished by the SGA. Based on participant report of

number of bowel surgeries, the majority of patients had no previous bowel surgeries. In the subgroup who had previously undergone bowel resection, the mean number of surgeries was 1.7.

Table 2. Participant demographics and disease characteristics

Sex	Male	22 (49%)
	Female	23 (51%)
Number of bowel surgeries	0	25 (56%)
	1	11 (24%)
	2	4 (9%)
	3	5 (11%)
Disease Status	Remission (CDAI <150)	27 (60%)
	Active (CDAI ≥150)	18 (40%)
Nutritional Status	Well Nourished (SGA-A)	30 (67%)
	Mild-Moderately Malnourished (SGA-B)	15 (33%)
	Severely Malnourished (SGA-C)	0 (0%)
Location of Inflammation	Small Bowel Only	17 (38%)
	Colon Only	10 (22%)
	Small Bowel + Colon	18 (40%)
	Upper GI Tract	0 (0%)
Albumin (g/L)	≥35 (normal)	37 (82%)
	<35 (low)	8 (18%)
	Mean	37.48 ± 3/7
CRP (mg/dL)	≤8 (normal)	36 (80%)
	>8 (high)	9 (20%)
	Mean	9.23 ± 22

4.3 Statistical Analysis

4.3.1 Active Disease Vs.. Remission

In patients with active disease (CDAI ≥150), the mean CRP was 16.1 mg/dL (±33.6) as compared to those in remission (CDAI<150), where the mean CRP was 4.7 mg/dL (±5.0). In active disease, the mean albumin was 36.3 g/L (±3.9) compared to 38.4 g/L (±3.3) in remission. These differences did not quite reach statistical significance using independent sample t-tests. The results are summarized in Table 3.

Table 3. Differences in active disease vs. remission

Variable	Active Disease	Remission	Difference	P-Value Independent sample t-test
CRP mg/dL (Normal 0-8)	16.1 (±33.6)	4.7 (±5.0)	11.4	0.088
Albumin g/L (Normal 35-50)	36.3 (±3.9)	38.4 (±3.3)	2.17	0.052

4.3.2 Well - Nourished vs. Mild-moderately malnourished

In patients who were well nourished (SGA-A) the mean CRP was 9.6 mg/dL (±25.3) while the mean CRP was 8.5 mg/dL (±13.8). in those who were mild-moderately malnourished (SGA-B). This difference was not statistically significant. In patients who were well nourished the mean albumin was 38.7 g/L (±3.17) while the mean albumin was 35.4 g/L (±3.76) in those who were

mild-moderately malnourished. This difference was statistically significant by independent sample t-test. These results are summarized in Table 4.

Table 4. Differences in SGA Class A vs. B

Variable	SGA-A	SGA-B	Difference	p-Value Independent sample t-test
CRP mg/dL (Normal 0-8)	9.6 (±25.3)	8.5 (±13.8)	1.1	0.88
Albumin g/L (Normal 35-50)	38.7 (±3.17)	35.4 (±3.76)	3.3	0.004

4.3.3 Serum albumin in well-nourished and malnourished groups with and without active inflammation

In well nourished (SGA-A) individuals with active inflammation (elevated CRP), the mean albumin was 35.8 g/L (±2.59), whereas in well-nourished patients without active inflammation (normal CRP) the mean albumin was 39.2 g/L (±2.99). This difference of 3.44 g/L was statistically significant using the student's t-test ($p = 0.024$). In these patients who were well nourished, the mean serum albumin was within the normal range (35 g/L to 50 g/L)[65] regardless of whether there was active inflammation as measured by CRP.

In contrast, patients who were both malnourished (SGA-B) and had active inflammation (elevated CRP) were, on average, hypoalbuminemic with a mean serum albumin of 31.5 g/L (±3.32). In patients who were malnourished (SGA-B) without active inflammation (normal CRP) the mean albumin was 36.8 g/L (±2.86), which is within the normal range. In malnourished

individuals with active inflammation the mean serum albumin was 5.32 g/L lower than the mean serum albumin in malnourished individuals without active inflammation. This difference was statistically significant with the student t-test ($p = 0.009$) as shown in Table 5.

These differences were also examined using Analysis of Variance (ANOVA) which showed a statistically significant difference in albumin between the well-nourished and malnourished groups ($p = 0.005$) and between the normal CRP and elevated CRP groups ($p < 0.001$). In linear regression, the interaction term SGA*CRP did not reveal a statistically significant interaction ($p = 0.408$), implying the effects are independent. Table 6 demonstrates these results.

Table 5. CRP and Albumin in well-nourished and malnourished groups

SGA	CRP mg/dL	Mean albumin g/L	Difference g/L Student's t-test	p-Value
Malnourished	Normal	36.8 (±2.86)	5.32	0.009
	Elevated	31.5 (±3.32)		
Well Nourished	Normal	39.2 (±2.99)	3.44	0.024
	Elevated	35.8 (±2.59)		

Table 6. Serum Albumin (g/L) by nutritional status and presence or absence of inflammation

Albumin (g/L) Normal 35-40	**Normal CRP (0-8 mg/dL)**	**Elevated CRP (>8 mg/dL)**	
Malnourished	39.2 (±2.99)	35.8 (±2.59)	Significance – ANOVA p = 0.005
Well Nourished	36.8 (±2.86)	31.5 (±3.32)	
	Significance – ANOVA P <0.001		

Among malnourished individuals - in those without inflammation (normal CRP) 9/11 (82%) have normal albumin, while in those with active inflammation, 0/4 (0%) have normal albumin (Fisher's exact test p = 0.011). In individuals who are well nourished - normal albumin is seen in 24/25 (96%) of those who have no inflammation and 4/5 (80%) of those with active inflammation (Fisher's exact test p = 0.31). This is demonstrated in Table 7.

Table 7. Proportion of patients with normal serum albumin levels by inflammation and nutritional status

	Serum Albumin		
Nutrition/CRP	Normal (%)	Low (%)	Fisher's Exact
Well-nourished/Normal (n=25)	24 (96)	1 (4)	p = 0.31
Well-nourished/ Elevated (n=5)	4 (80)	1 (20)	
Malnourished/Normal (n=11)	9 (82)	2 (18)	p = 0.011
Malnourished/Elevated (n=4)	0 (0)	4 (100)	

4.3.4 Nutritional Status and Disease Status

To examine the relationship between active disease and nutritional status (Table 8) a chi-squared test was used, which demonstrated a Pearson Chi-Square value of 6.667, Pearson's R-value of 0.385, and an asymptotic significance of 0.010. Fisher's Exact test confirms significance with a two-sided significance of 0.022. The odds ratio of being well nourished in remission was 5.5 (95% CI 1.43-21.1).

Table 8. Active disease by nutritional status

	Well Nourished SGA-A	**Mild-Moderately Malnourished SGA-B**	**Total**
Remission (CDAI<150)	22 (49%)	5 (11%)	27 (60%)
Active Disease CDAI≥150)	8 (18%)	10 (22%)	18 (40%)
Total	30 (67%)	15 (33%)	OR 5.5 (95% CI 1.43-21.1)

4.3.5 Correlation between study variables

Linear correlations were assessed using the Pearson correlation coefficient for continuous variables and Spearman's rank order correlation coefficient for categorical data. Variables with skewed distributions (CRP and CDAI) were log transformed. There was a statistically significant negative correlation between nutritional status as measured with the SGA and serum albumin with a Spearman's rank order correlation coefficient of -0.406 ($p = 0.006$). There was also a statistically significant negative association between the degree of inflammation as measured with Log CRP and serum albumin with a Pearson correlation coefficient of -0.44 ($p = 0.002$). There was a

statistically significant negative correlation between both disease activity (measured with Log CDAI) and serum albumin with a Pearson correlation coefficient of -0.415 (p = 0.005) and nutritional status with a Spearman rank correlation coefficient of 0.474 (p = 0.001). Log CDAI and Log CRP were also significantly correlated with a Pearson coefficient of 0.376 (p = 0.011). These results are summarized in Table 9.

Table 9. Simple Correlation

		LogCDAI	LogCRP	Albumin	SGA*
LogCDAI	Pearson Correlation		0.376	-0.415	0.474
	Significance (2 Tailed)		0.011	0.005	0.001
LogCRP	Pearson Correlation	0.376		-0.44	-0.02
	Significance (2 Tailed)	0.011		0.002	0.90
Albumin	Pearson Correlation	-0.415	-0.44		-0.406
	Significance (2 Tailed)	0.005	0.002		0.006
SGA*	Spearman Correlation	0.474	-0.02	-0.406	
	Significance (2 Tailed)	0.001	0.90	0.006	

*Spearman's rank order correlation coefficient

4.3.6 Multiple Linear Regression

Multiple linear regression was carried out to examine the independent effects of inflammation (LogCRP) and nutritional status (SGA) on serum albumin. Both LogCRP ($p = 0.001$) and SGA ($p = 0.002$) were statistically significant in the regression analysis. Age and sex were added to the analysis, which was limited to four variables due to our sample size of 45 participants. Both LogCRP and SGA remained significant when age and sex were added to the analysis. In the regression analysis, the R for LogCRP and SGA was 0.605, with an adjusted R^2 of 0.336 and a significance of 0.002, which would indicate that 34% of the variance in albumin in our model can be explained by LogCRP and SGA. The regression equation for this model was serum albumin = 39.807 - 4.33(LogCRP) - 4.15(SGA).

Chapter 5: Discussion

This cross-sectional study set out to further explore the complexities of hypoalbuminemia in Crohn's disease – specifically the association between both inflammation and malnutrition. Ultimately, there were independent moderate correlations between hypoalbuminemia and malnutrition (measured by SGA) as well as hypoalbuminemia and inflammation (measured by CRP). This suggests that both inflammation and malnutrition play a role in the hypoalbuminemia that is seen in the Crohn's disease population.[55,56]

Participants without active inflammation were more likely to be well-nourished. However, there were some participants who were malnourished with no inflammation and some who were well-nourished despite active inflammation. When compared to those who were well-nourished with no active inflammation, serum albumin levels were seen to be lower in those with only active inflammation or only malnutrition. However, hypoalbuminemia was more pronounced in those who had both inflammation and malnutrition.

As the main characteristic of IBD, inflammation is routinely assessed using multiple methods in this population. Hypoalbuminemia in these patients is often seen alongside laboratory tests and endoscopic investigations that are more specific for inflammation. If seen in isolation, hypoalbuminemia could prompt further assessment of inflammation in these patients.

Despite literature in non-diseased individuals suggesting that hypoalbuminemia does not occur until the extremes of starvation, we suggest that there may be an underlying process in individuals with active IBD that leads to an increased tendency toward hypoalbuminemia when any degree of malnutrition is present. As such, a low threshold for more robust nutritional

assessment would be reasonable in patients with Crohn's disease and any degree of hypoalbuminemia. In individuals with known active inflammation that may be thought to explain hypoalbuminemia, it would be warranted to pursue nutritional assessment with the SGA or other validated tools if hypoalbuminemia is identified, especially in severe cases.

5.1 Participant Characteristics

5.1.1 Sex

Our population included similar male to female representation (49:51). This is not typical of the population with Crohn's disease in North America overall, which has a female preponderance with a female: male ratio from 1.2 to 1.5.[55,56] As male sex has been identified as a risk factor for more severe disease, a sample such as ours with a disproportionate number of male participants may represent patients with more active disease, malnutrition, and increased number of surgeries.

5.1.2 Age

The mean age of participants in our study was 51.5 years. This is in keeping with American prevalence data, which found an average age of 50 years among individuals with Crohn's disease.[33] The largest Canadian epidemiologic study, by Bernstein et al in 2006, does not report an average age of individuals with Crohn's disease. Instead, the prevalence is reported by age group, with the highest prevalence shown in those aged 30-39. Our population skews older than this, as the highest proportion of participants was between age 40-49. In 2006, the average age of a Canadian was 38.8, whereas in 2020 the average age of a Canadian was 41.4 years.[69] Beyond that, the population of Newfoundland and Labrador is, on average, the oldest in Canada with an

average age of 44.8 in 2020.[69] The older age of the population of our study may then be expected given the aging Canadian population and the slightly older population of Newfoundland and Labrador.

5.1.3 Disease Activity

At the time of study, 60% of participants were in clinical remission based on CDAI. Similar Canadian studies have found that, at a single point in time, approximately 60% of CD patients will have disease in remission as observed in our population.[29,70] This is compatible with the documented natural history of CD as a relapsing and remitting disease that has been shown to have ~55% of patients in remission, 30% with highly active disease, and 15% with mildly active disease.[33,71] Of the patients included in this study, 44% had undergone surgeries related to their disease. This is in keeping with both Canadian[72] and American[73] literature that demonstrated that 48% and 46.6%, respectively, of CD participants had required bowel surgery.

5.1.4 Location of Inflammation

In our study population, 22% of patients had isolated colonic inflammation, 40% had small bowel and colonic inflammation, and 38% had isolated small bowel inflammation. In a single centre Canadian cohort in 2001, 27% of patients had colonic inflammation, 34% had ileo-colonic inflammation, 25% had inflammation of the terminal ileum, and 12% had a combination of colonic or ileal inflammation and upper GI inflammation.[74] Other studies reflect a range of 23% to 48% of patients with colonic inflammation, 14% to 28% with ileal inflammation, and 22% to 60% with ileo-colonic inflammation. [73–77] Given this range and the available Canadian data, the distribution

of inflammation seen in our population is likely adequately representative of the NL CD population.

5.2 Nutritional Status

5.2.1 Overall Nutritional Status

The SGA was used to determine nutritional status in all participants in the study. The decision to use the SGA was based on the validation of this scale as a diagnostic, rather than screening, test for malnutrition.[12,23] In the population as a whole, 30/45 participants (67%) were found to be well-nourished, while 15/45 (33%) were found to have mild-moderate malnutrition. No patients were found to be severely malnourished.

Previous research on the nutritional status of patients with IBD has reported wide variation in the prevalence of malnutrition. Our study demonstrated a much higher rate of malnutrition than the 8% prevalence seen in CD patients in a 2017 multi-centre epidemiologic study by Casanova which also utilized the SGA to measure the nutritional status of patients with IBD.[31] However, nutritional assessment in their study was completely voluntary and was performed on only 25% of patients with CD. It is therefore difficult to determine if the rate of malnutrition seen in their study is representative of the Crohn's disease population as a whole. Furthermore, only 27% of participants in their study had active disease, and 88% of those had mild disease.[31] As reviewed above, the estimated rate of active disease in the CD population is approximately 40%.[29,70] Our population had a higher rate of active disease than those in the Casanova study, which more accurately represents the anticipated prevalence of disease activity in the Canadian population. This may have contributed to the higher rate of malnutrition seen.

In contrast, Mijac et al (2010) used multiple assessment methods to determine the prevalence of malnutrition in patients with IBD. Overall, they found a rate of malnutrition that varied from 69% as determined by mid arm circumference to 25% when determined by body fat percentage as measured by the sum of four skinfold assessments.[30] Cabral et al (2017) similarly found a high rate (78%) of malnutrition in patients with CD using anthropometric measures.[56] Neither study used any composite scores in their diagnosis of malnutrition, and they did not utilize the SGA in their assessment, so it is difficult to compare their findings to our population, but they do demonstrate high rates of malnutrition in patients with IBD.[30,56]

Overall, 33% is a relatively high prevalence of malnutrition and perhaps higher than we anticipated. It was, however, mild-moderate malnutrition with no cases of severe malnutrition found. Based on the previously published prevalence rates, it is difficult to accurately determine if this is higher than should be expected in the Crohn's disease population, as the prevalence of malnutrition seems to vary significant depending on the instrument used.

5.2.2 Nutritional status and Crohn's disease activity

A moderate correlation of 0.474 was found between nutritional status (SGA) and Crohn's disease activity. This was also confirmed using categorical variables for both nutrition (SGA-A vs. B) and disease activity (active disease vs. inactive disease based on CDAI). This is in keeping with previous literature that documents an association between active disease and poor nutrition in patients with Crohn's disease.[78]

It is possible that some degree of the association between the SGA and the CDAI may be in part due to common subjective components that the two measures share. However, both of these

scales can be "positive" without the components that they have in common, and both are validated as independent scores so it was felt that they could be analyzed separately.

5.2.3 Nutritional status and inflammatory markers

Our study found no significant correlation between nutritional status (SGA) and inflammation as measured by logCRP. This is in contrast to a 2016 study by Jensen at al that found a "small correlation" between the SGA and CRP in individuals with inflammatory bowel disease. The strength of the correlation was not reported in their study, as this specific correlation was not the primary outcome that was being measured.[79]

Our findings are inconsistent with the available literature on the correlation between CRP and the SGA in patients with chronic kidney disease. Essadik et al documented a moderate to strong association between SGA category and CRP ($r = 0.65$, $p = 0.04$) in patients with chronic kidney disease.[57] Similarly, Qureshi et al found a significant difference in serum CRP in individuals with severe malnutrition (SGA-C) compared to those who were well nourished (SGA-A) or mild-moderately malnourished (SGA-B).[59] In Qureshi et al's study, elevated CRP was seen to be due to infection in the majority of cases (17/22).

Serum levels of CRP rise and fall relatively rapidly in the setting of active inflammation, with clinical elevation within 6 hours after an initial stimulus, a peak level approximately 48 hours after initial stimulus, and a half-life of 19 hours.[80] In contrast, clinical signs of malnutrition can take significant time to develop and to resolve. This difference may have contributed to the lack of correlation seen in our study as malnutrition related to inflammation may persist even after the active inflammation has resolved. Our study also did not have any individuals with severe

malnutrition (SGA-C). It is possible that the correlation between inflammation and malnutrition that is documented in other studies would be seen in more severe states of malnutrition. Finally, it may be that our study was underpowered to demonstrate a lack of correlation, which would require a larger sample size.

5.2.4 Limitations of Nutritional Assessment

The SGA has been shown to have higher intra-rater reliability than inter-rater reliability. In our study, a single researcher administered the SGA rather than multiple assessors across the sample. This researcher was trained in appropriate physical exam techniques and assessment of nutritional status and was not made aware of the patients' biochemical markers prior to the assessment. With only one assessor for each patient, there was a potential for bias if that assessor tended to systematically give higher or lower scores than appropriate on the subjective components of the SGA. This was mitigated by using an assessor with previous training in physical exam techniques who had reviewed multiple SGA training scenarios to compare with previously determined scores. As the SGA is a validated tool for single assessor use, this was felt to provide appropriate assessment of nutritional status. It would not be feasible to have multiple assessments of each patient's SGA with the constraints of a clinic appointment and ongoing regard for the participant's time.

Some components of the SGA rely heavily on participant recollection, rather than objective findings. For some participants, an estimate of their general nutritional intake over an extended period (6 months) was difficult to obtain. This subjectivity to the SGA could lead to recall bias, which could result in both type I or type II errors in the diagnosis of SGA, depending on individual factors in how these questions are answered. Thorough food monitoring or review with a dietitian

may be beneficial in patients who have a difficult time with recollection or estimation of intake over such a time period.

The strong association between nutritional status and disease activity seems to be seen in studies that use the SGA as a measure of nutritional status, but not in those that use anthropometric measures.[30,31,56,78] The SGA section on "symptoms" is targeted at symptoms that can cause decreased oral intake due to difficulty or discomfort with eating. Nausea, pain with eating, diarrhea, vomiting, and anorexia are all included in the SGA and have significant overlap with symptoms of active Crohn's disease. While this category contributes to the SGA score, identification of these symptoms alone will not lead to a diagnosis of malnutrition. There must be other, more objective, contributors involved in the scoring of the SGA to lead to a diagnosis of even mild malnutrition.[12] Because of this, it was felt that the SGA and the CDAI could be analyzed separately, despite some symptoms in common (namely diarrhea and abdominal pain).

Nonetheless, it is possible that the strong association seen between nutritional status and disease activity in our study is influenced by the features that they have in common. In any study examining nutrition and CD this is likely to be a potential issue, as the symptoms of active disease tend to be the same symptoms that can lead to malnutrition and, therefore, are used as surrogate markers in composite measures for malnutrition. The addition of anthropometric measures in addition to the SGA could be considered to mitigate potential confounding.

5.3 Inflammation

5.3.1 Severity of inflammation

The CDAI characterizes disease activity into three categories. CDAI scores <150 represent remission, scores from 150 - 449 represent active disease, and scores ≥ 450 represent severe disease. Our study included no participants from the severe disease category, with the highest CDAI score among our population being 361.[48] Patients with severe disease are more likely to be seen in the emergency department or as hospital inpatients. As our study was limited to the ambulatory care setting, the likelihood of including the population with severe disease is decreased. This could be explored in future research with this population to ensure that disease severity is representative of the CD population as a whole.

5.3.2 Inflammation and Disease Activity

In our study, the C-reactive protein was used to measure inflammation in patients with Crohn's disease. Patients with active disease based on a CDAI ≥150 had an average CRP of 16.1 mg/L vs. 4.7 mg/L in patients who were in remission, a difference that did not ultimately reach statistical significance. Various cutoffs have been used in studies of inflammatory markers in Crohn's disease with values greater than 8 mg/L-10 mg/L correlating with endoscopic or clinical disease activity.[81,82] Clinically, a CRP of 16.1 would generally be considered "abnormal" while a CRP of 4.7 would be considered "normal." The difference in CRP between patients with active disease and those in remission has been shown across the literature and the difference we demonstrated may be clinically significant. It is possible that with a larger sample size, this

difference would have reached statistical significance, though this was not the primary outcome our study was powered for.

5.3.3 Limitations of C-reactive protein

Elevated CRP is, unfortunately, non-specific. It can arise from multiple etiologies, such as an active infection or other inflammatory conditions that can be associated with Crohn's disease, such as arthritis. One limitation of our study is that we did not control for concurrent infections or other inflammatory events around the time of blood draws. Similarly, inflammatory arthritis was not an exclusion criterion for our study due to its frequency in patients with Crohn's disease. This is a limitation of the use of CRP to measure inflammation in this population, as participants may have had elevated CRP for reasons other than activity of their bowel disease.

It has also been shown that up to 25% of individuals with active Crohn's disease on endoscopy do not have an increased level of CRP. The pathophysiology of this is thought to be multifaceted and may include: the degree of penetration of inflammation into deeper layers of the bowel wall; the location of inflammation; and genetic factors contributing to CRP expression.[75,83,84] As a result, some individuals with inflammatory bowel disease will not express CRP despite active inflammation, which can lead to an increase in type II errors, or false negatives.

Erythrocyte sedimentation rate (ESR) is another inflammatory marker that has been used to measure disease activity in CD. The literature suggests that ESR is as equally non-specific as CRP. It is also significantly more costly to the health-care system and has fallen out of favor in recent years. Using ESR in conjunction with CRP may have been more sensitive to inflammation

in CD patients in this study, but would not have improved the specificity and was not an available option for routine analysis as ESR is not routinely measured in our IBD population.[49,81]

The use of fecal calprotectin as a measure of inflammation in Crohn's disease patients has increased significantly in the last decade. As a stool test, it measures inflammation that is specific to the GI tract. The use of fecal calprotectin to assess inflammation in this study would have been more specific than CRP. Fecal calprotectin is more likely to be elevated in patients with distal (colonic) inflammation, so patients with proximal (i.e.small bowel or upper GI) CD may have a negative fecal calprotectin despite inflammation in their bowel.[50,51] These patients may represent a different subgroup than those who would have false negative CRP. There can be difficulties with reliability of fecal sample collection, and at the point of our study it was much more likely that patients would have had CRP measured for routine outpatient care than fecal calprotectin. As such, it was felt that fecal calprotectin was not a viable option for this study.

Overall, CRP has been validated as a marker of inflammation in Crohn's disease.[75] There are certainly some limitations to its use, but it was felt that it was the most representative of current practice for monitoring inflammation in patients with CD. In a prospective study, or differently designed cross sectional study, a combination of fecal calprotectin and CRP would likely be a more accurate representation of inflammation in CD patients than CRP alone and might decrease the potential for both type I and type II errors.

5.4 Hypoalbuminemia

This research project set out to explore the factors contributing to hypoalbuminemia in Crohn's disease, with the foundational premise that low albumin is correlated with disease activity

due to the effects of both inflammation and malnutrition. In order to investigate this, we used simple correlation and multiple regression to explore the correlation between albumin and inflammation, disease activity, and nutritional status.

5.4.1 Serum albumin and nutritional status

There was a statistically significant negative correlation between serum albumin and nutritional status as measured with a Spearman rank correlation. When nutritional status was categorized into "SGA - A" or well-nourished and "SGA - B" or mild-moderately malnourished, there was a statistically significant difference in average serum albumin between groups. These results demonstrate that albumin has a negative correlation with nutritional status, with serum albumin lower in individuals who are malnourished as compared to those who are well nourished.

Significant correlation between nutritional status and albumin has not been seen in non-diseased malnourished patients until they reach the extremes of starvation.[28] Given the intricacies of albumin synthesis and metabolism, it may be that patients with inflammatory diseases are more susceptible to hypoalbuminemia in less severe states of malnutrition than non-diseased individuals. In patients with IBD, there is limited research that explicitly addresses this question.

The negative correlation between albumin and nutritional status was not found in Cabral's study examining the role that albumin plays in IBD. Their study used anthropometric data to establish a diagnosis of malnutrition, and they found a significantly higher proportion of patients with IBD to be clinically undernourished (77.8%). This high prevalence of malnutrition is out of keeping with other studies of nutritional status in IBD. Given the difference in method of

assessment of nutritional status between our research and that used in the Cabral study, it is difficult to compare results between the two.[56]

In similar literature on serum albumin and malnutrition in CKD, there was a significant correlation between albumin and nutritional status when measured by the SGA.[34,57,59] This correlation was not seen in studies where nutritional status was measured by anthropometric measures alone.[58] Within studies that measured nutritional status by both the SGA and anthropometric data, the association with albumin was only observed with the SGA.[34,57–59]

5.4.2 Serum albumin and inflammation

There was a moderate negative association between albumin and LogCRP that was statistically significant. This finding is in keeping with the strong association seen between albumin and inflammation in studies that include CKD patients. Other studies examining the relationship between albumin and inflammation in IBD have found a significant, but small, negative correlation between albumin and inflammatory markers.[1,2,55]

Given the physiologic role of albumin as a negative acute phase reactant, it would be expected for albumin and inflammation to have a significant negative correlation. Cabral's study found a correlation between inflammation and albumin as well as inflammation and disease activity using ESR, rather than CRP as a surrogate marker of inflammation.[56] As outlined above, there are a number of other measures available to assess inflammation in IBD. It is plausible that the inclusion of other markers of inflammation, such as ESR or Fecal Calprotectin, would yield similar results in the association between albumin and inflammation.

5.4.3 Serum albumin and disease activity

There was a statistically significant negative association between albumin and Crohn's disease activity, which is in keeping with the negative correlation identified in previous literature.[1,2,32,33,35] When disease activity was classified into "active disease" and "remission" based on CDAI, the difference in mean albumin level did not quite reach statistical significance. As continuous outcomes can more easily demonstrate associations, the lack of statistical significance once the outcomes were dichotomized is perhaps to be expected. The observed difference would, again, be similar to previous literature documenting the negative association between albumin and disease activity in inflammatory bowel disease.

5.4.4 Serum albumin, inflammation, and nutritional status

Hypoalbuminemia is relevant in CD because it is associated with disease activity. We have also observed a negative correlation between albumin and both inflammation (as measured by CRP) and malnutrition (as measured by SGA). In order to confirm and further explore the independent effects of inflammation and malnutrition on serum albumin, we performed multiple linear regression analysis of serum albumin vs. SGA and LogCRP. Both LogCRP and SGA were statistically significant in the regression analysis, which indicates that both inflammation and malnutrition are associated with hypoalbuminemia independent of one another in patients with Crohn's disease. Both factors remained highly significant when age and gender were added to the regression equation. There was no indication of any significant interaction between the variables through ANOVA or with the addition of an interaction term into the multiple regression.

Overall, the correlation between serum albumin and disease activity documented in previous literature was confirmed with simple correlation. Our study set out to explore this relationship and determine if the relationship between serum albumin and disease activity was driven primarily by inflammation or malnutrition by assessing the correlation between albumin and these two factors. Serum albumin was found to be moderately associated with nutritional status as measured by the SGA, inflammation as measured by CRP, and with disease activity as measured by CDAI.

The mean serum albumin was low in malnourished individuals with active inflammation by CRP, whereas the mean serum albumin was normal in those who were malnourished without active inflammation by CRP. Conversely, in individuals who were well nourished, the mean serum albumin was within the normal range in those with and without active inflammation as measured by CRP. Similarly, 82% of individuals with malnutrition and no inflammation had normal albumin, while none with malnutrition and inflammation had normal albumin. This difference was statistically significant. In groups who were well nourished, there was no statistically significant difference in the proportion of individuals with normal albumin in those with or without active inflammation.

This is in keeping with the hypothesis that individuals without underlying inflammatory processes who develop malnutrition are unlikely to develop hypoalbuminemia until the extremes of starvation but those who have underlying inflammatory disorders may have a higher likelihood of developing hypoalbuminemia at earlier stages of malnutrition.[28] The analysis of these subgroups involved small sample sizes. As such, Fisher's exact test was used in the analysis. A larger sample size may be beneficial in future research to investigate this relationship further.

5.5 Limitations

5.5.1 Limitations with Sample Size

Initial sample size calculations used a predicted correlation of 0.28 between albumin and inflammation and a predicted correlation of 0.37 between albumin and nutritional status based on previously documented associations in CKD. In order to detect a correlation as low as 0.28 with the type I error rate set at 0.05 and the type II error rate set at 0.20, the predicted sample size was calculated to be 95. Recruitment for this study was stopped at 45 participants due to the limitations in research during the COVID-19 pandemic. A post-hoc sample size calculation was performed. With a sample size of 45, a correlation coefficient as low as 0.4052 can be detected with the type I error rate set at 0.05 and the type II error rate set at 0.20.[60] The observed correlation in our study was -0.44 between albumin and inflammation (LogCRP) and -0.406 between albumin and nutritional status. Both of these observed correlations are above the predicted correlations and the study remained appropriately powered despite the lower than anticipated sample size. In future study, a larger sample size would allow for the analysis of more potential confounders.

5.5.2 Selection Limitations

This study was a single centre study in a tertiary care centre. Participants were routinely followed by gastroenterologists (subspecialists) in a hospital affiliated clinic. This could limit external validity by creating a sample that may not be representative of the Crohn's disease population as a whole and may have included patients with more severe inflammatory bowel disease and more significant malnutrition than patients with less severe disease managed in the community. Conversely, management by a gastroenterologist in this setting can result in a sample

with disease under better control due to the specialized knowledge base. We did not specifically assess disease severity in terms of extent of disease or presence of complications such as fistulizing disease and extraintestinal manifestations. The fact that, by CDAI, 60% of patients were in remission does suggest our sample may have been representative.

5.5.3 Limitations with measurements

Given the nature of this study, biochemical markers were used if they had been collected within 1 month of in person examination. It is possible that CRP or albumin values drawn 1 month apart from the SGA would be discrepant to the values that would have been drawn on the day of administration. This is especially true in the case of patients who had intercurrent illnesses or changes in management. A larger sample size may have allowed for subgroup analysis of the effect that this timing had.

5.6 Conclusion

Albumin has been shown to be a marker of both malnutrition and inflammation in various disease states but there has been very little research into the precise etiology of hypoalbuminemia in Crohn's disease. The relationship between albumin and disease activity in Crohn's disease is complex. Malnutrition, a common complication of Crohn's disease may lead to hypoalbuminemia. Inflammation, an inherent component of active Crohn's disease may also lead to low albumin levels. Protein losing enteropathy may also contribute to hypoalbuminemia in Crohn's disease.

In our study, hypoalbuminemia was independently associated with both malnutrition and inflammation in patients with Crohn's disease but was most profound in subjects with both malnutrition and active inflammation. These results suggest that although a low serum albumin in

Crohn's disease may be a marker of either malnutrition or active disease, the lowest levels will be seen in patients with both. Future study could be undertaken to identify the utility of albumin to prompt further investigation into active inflammation as well as the effect of nutritional interventions on those with inflammatory bowel disease and hypoalbuminemia.

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Appendices

Appendix 1: Subjective Global Assessment

Adapted from Detsky et al 1987

Subjective Global Assessment			
Nutrient Intake			
1. ☐ No change; Adequate			
2. Inadequate	Duration of inadequate intake _____		
☐ Suboptimal solid diet	☐ Full fluid/oral nutritional supplen	☐ Minimal intake, clear fluid/starvation	
3. Nutritional intake in past 2 weeks			
☐ Adequate	☐ Improved but not adequate	☐ No improvement or inadequate	
Weight	Usual Weight _____	Current Weight _____	
1. Non fluid weight change in past 6 months		Weight loss (kg) _____	
☐ <5% loss or stable		☐ 5-10% without stabilization or increase	
If above not known, has there been a subjective loss of weight during the past 6 months?			
☐ None/Mild	☐ Moderate	☐ Severe	
2. Weight change past 2 weeks		Amount (if known) _____	
☐ Increased	☐ No change	☐ Decreased	
Symptoms			
1. ☐ Pain on eating	☐ Anorexia	☐ Vomiting	☐ Nausea
☐ Mouth pain	☐ Early satiety	☐ Constipation	☐ Dysphagia
2. ☐ None	☐ Mild/Few	☐ Severe/Multiple	
3. Symptoms in past 2 weeks			
☐ Resolution	☐ Improving	☐ No change or worse	
Functional Capacity			
1. No dysfunction			
2. Reduced capacity (duration _____)			
☐ Difficulty with ambulatio	☐ Immobility		
3. Functional Capacity in past 2 weeks			
☐ Better	☐ No change	☐ Worse	
Metabolic Requirement			
High metabolic requirement?			
☐ Yes	☐ No		
Physical Exam			
Loss of body fat			
☐ No	☐ Mild-mod	☐ Severe	
Loss of muscle mass			
☐ No	☐ Mild-mod	☐ Severe	
Presence of edema/ascites			
☐ No	☐ Mild-mod	☐ Severe	
TOTAL SGA			
☐ A	☐ B	☐ C	
Well nourished/normal	Mild/mod malnourished	Severely malnourished	
Contributing Factor			
☐ Cachexia	☐ Sarcopenia		

Appendix 2: Crohn's Disease Activity Index

Adapted from Best et al 1976[48]

Crohn's Disease Activity Index	
Number of liquid or soft stools each day over past week	x2 = _____
Abdominal pain (0-3 each day for 7 days)	x5 = _____
General well being (0-4 each day for 7 days)	x7 = _____
Presence of complications	x20 = _____
Anti diarrheals	x30 = _____
Presence of abdominal mass (0,2,5)	x10 = _____
Hematocrit <0.47 or <0.42 (M:F)	x6 = _____
Deviation from standard weight (%)	x1 = _____
Arthralgia/arthritis	1/0 _____
Iritis/uveitis	1/0 _____
Erythema nodosum/pyoderma gangrenosum, apthous ulcers	1/0 _____
Anal fissures/fistulae/abscess	1/0 _____
Other fistulae	1/0 _____
Fever in past 7 days	1/0 _____
	Total = _____

Appendix 3: **Conceptual Framework** – The relationship between malnutrition, albumin, and inflammation in patients with IBD

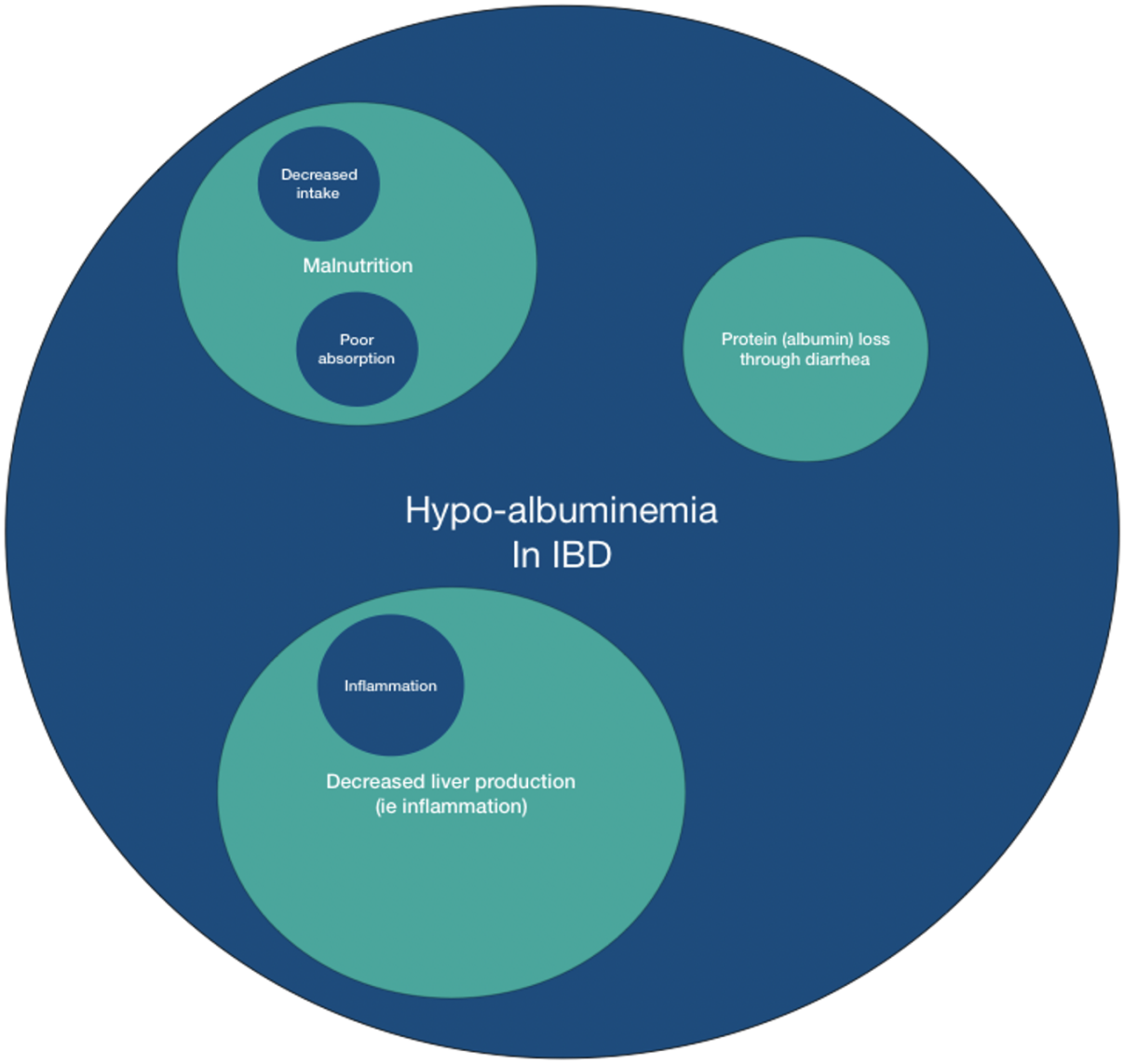

Figure A1: Conceptual framework for research of hypoalbuminemia in IBD. Various etiologies contribute to hypoalbuminemia in these patients, and there are various physiologic factors about the disease that contribute to these areas.

Appendix 4: Ethics Approval

NEWFOUNDLAND AND LABRADOR
HREB
HEALTH RESEARCH ETHICS BOARD

Research Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

June 05, 2019

Dear Dr Squirell:

Researcher Portal File # 20192985
Reference # 2019.058

RE: Serum Albumin as a Measure of Inflammation or Malnutrition? A Cross Sectional Study

Your application was reviewed by a subcommittee under the direction of the HREB and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective June 5, 2019. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Letter to the gastroenterologists, approved
- Letter explaining the process of sharing the SGA and CDAI with the GI if patients opt to choose to share this information, approved
- Informed Consent, approved
- Data Custodian Albumin, approvoed
- CDAI, screen shot, approved
- Subjective global assessment, approved
- Research proposal, approved

Please note the following:

- This ethics approval will lapse on June 5, 2020. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.
- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

We wish you every success with your study.

Sincerely,

Dr E Dicks, Co-Chair, Non Clinical Trials
Health Research Ethics Board