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Anemia in Pediatric Intestinal Failure: Prevalence, Predictors and Etiologies

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Prevalence, Predictors and Etiologies

by

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Abstract

Children with intestinal failure (IF) are at high-risk for different types of anemia, including iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed IDA/AI. Data on the prevalence and underlying contributors to the types of anemia in the pediatric IF population is limited. Therefore, the aim of this thesis was to examine the prevalence and contributions of the various types of anemia in children with IF and identify factors associated with these anemias.

A 10-year retrospective, multicenter study of pediatric IF patients managed by three separate intestinal rehabilitation programs (IRPs) in Canada was conducted. Anemia was defined by age-specific hemoglobin values, and anemia types were classified using a combination of hematologic measures and iron indices. Univariable regression analysis was performed to evaluate for demographic and clinical factors associated with anemia and anemia types.

Among ninety children with IF, the period prevalence of anemia was 83% [75/90], with 76% [55/72] of children experiencing chronic anemia, defined as anemia on ≥ 2 annual hemoglobin measurements. AI (44%) [40/90] and mixed IDA/AI (36%) [32/90] were more prevalent than IDA (17%) [15/90]; 26% [19/90] children developed >1 type of anemia over time, and 84% [191/227] of anemic hemoglobin measurements occurred while receiving iron supplementation, oral or in parenteral nutrition (PN). The prevalence of mixed IDA/AI was higher at 2 IRPs that did not have access to iron-supplemented PN (75% vs 9%; $p<0.001$), as was small intestine bacterial overgrowth (SIBO) (58% vs 28%; $p=0.004$) and gastrointestinal bleeding (39% vs 15%; $p=0.001$). Children receiving iron-supplemented PN had lower odds of mixed IDA/AI compared to no anemia (OR 0.06, $p<0.001$), while oral iron supplementation was associated with an increased odds of mixed IDA/AI compared to no anemia (OR 3.40, $p=.01$).

This study demonstrated a high prevalence of anemia in children with IF, specifically mixed IDA/AI and AI. This anemia is often chronic and dynamic with evolving anemia types. Our results suggest that mode of iron supplementation may impact IF-associated complications and anemia types. Future studies exploring the complex interactions between the gut microbiome, mode of iron supplementation and inflammation on anemia in pediatric IF are needed.

Preface

This thesis is original, unpublished, independent work by the author, Jaclyn Strauss. The results reported were covered by Ethics Certificate number REB21-0782 issued from the Conjoint Health Research Ethics Board (CHREB) at the University of Calgary (Calgary, Alberta)for the project "Anemia in Pediatric Intestinal Failure: Prevalence, Etiologies and Predictors" and REB21-0782 psite00000032 issued by the CHREB at the University of Alberta (Edmonton, Alberta). The results were also covered by Ethics Certificate number REB 1000079556 issued from the Research Ethics Board at SickKids Hospital (Toronto, Ontario).

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Abbreviations

Abbreviation	Definition
5-ASA	5-aminosalicylate
AI	Anemia of Inflammation
AU	Anastomotic Ulcer
CBC	Complete Blood Count
CFU	Colony forming units per milliliter
CHIRP	Children's Intestinal Rehabilitation Program
CKD	Chronic Kidney Disease
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
dL	deciliter
DCYTB	Duodenal Cytochrome B
DMT1	Divalent Metal Transporter 1
eGFR	Estimated Glomerular Filtration Rate
EGD	Esophagogastroduodenoscopy
EMR	Electronic Medical Record
EN	Enteral Nutrition
EPO	Erythropoietin
ESR	Erythrocyte Sedimentation Rate
FCP	Fecal Calprotectin
FPN1	Ferroportin 1
g	Gram
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GIFT	Group for Improvement of Intestinal Function

GJ	Gastro-jejunal
GT	Gastrostomy Tube
HCP1	Heme Carrier Protein 1
IBD	Inflammatory Bowel Disease
ICV	Ileocecal Valve
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IF	Intestinal Failure
IFALD	Intestinal Failure Associated Liver Disease
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	Interquartile Range
IRP	Intestinal Rehabilitation Program
IV	Intravenous
KIDGO	Kidney Disease: Improving Global Outcomes (KIDGO)
m^2	Meters squared
MCV	Mean Corpuscular Volume
mg	milligram
min	minute
mL	milliliter
MMA	Methylmalonic Acid
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
PN	Parenteral Nutrition
PPI	Proton-Pump Inhibitor

ppm	Parts per million
REB	Research Ethics Board
RBC	Red Blood Cell
SBS	Short Bowel Syndrome
SIBO	Small Intestine Bacterial Overgrowth
STEP	Serial Transverse Enteroplasty
sTfR	Soluble Transferrin Receptor
sTfR-F Index	Soluble Transferrin Receptor-log Ferritin Index = sTfR (mg/L)/log Ferritin (μg/L)
TIBC	Total Iron Binding Capacity
TNF-α	Tumor Necrosis Factor-alpha
TSAT	Transferrin Saturation
UC	Ulcerative Colitis
μg	microgram
μmol	micromole

1. Chapter 1: Anemia in Pediatric Intestinal Failure – A Literature Review

1.1 Pediatric Intestinal Failure (IF)

Pediatric intestinal failure (IF) is a devastating condition characterized by the inability of the gastrointestinal tract to absorb adequate macronutrients and water to sustain normal growth, development and health due to a reduction of functional intestinal mass.[1] The causes of pediatric IF can broadly be defined as being due to disorders of intestinal function, which includes motility disorders (*e.g*. Hirschsprung disease, chronic intestinal pseudo-obstruction) and mucosal enteropathies (*e.g*. congenital diarrhea disorders, tufting enteropathy), or disorders of insufficient bowel length. Short bowel syndrome (SBS), defined as the consequence of any natural loss or surgical resection of small bowel, is the leading cause of IF in children and is most often secondary to necrotizing enterocolitis (NEC), gastroschisis, congenital intestinal atresia or volvulus.[2] Using the Canadian Association of Paediatric Surgeons (CAPS) definition of SBS as the need for total parenteral nutrition greater than 42 days after bowel resection, or a residual small bowel length of less than 25% expected for gestational age, a Canadian population-based study in 2004 estimated the incidence of neonatal SBS to be 24.5 per 100 000 live births.[3] The resulting intestinal anatomy and bowel length in SBS predisposes individuals to specific micronutrient and vitamin deficiencies, many of which play key roles in gastrointestinal structure, function and adaptation.[4] Initially, all children with IF are dependent on parenteral nutrition (PN), nutrition received directly into a vein, for survival. While up to 50% will achieve adequate enteral autonomy and come off PN, this can take months to years to achieve. [3,5] Due to advances in care, including multidisciplinary intestinal rehabilitation programs, alternative PN formulations and innovative bowel lengthening procedures, the majority of children with IF are now surviving through

childhood and thus, new long-term complications and comorbidities are being identified.[6] Anemia is a frequent, yet poorly described complication in pediatric IF with considerable implications for long-term outcomes.

1.2 Anemia

1.2.1 Anemia – Definition and Types of Anemia

Anemia is defined as a reduction in the total number of red blood cells (RBCs) or hemoglobin concentration in the blood.[7] Acute signs and symptoms of anemia can include pallor, fatigue, dizziness, weakness, exercise intolerance and arrhythmias and in severe cases can lead to seizures, heart failure and death.[8,9] Anemia has considerable negative long-term consequences including associated impairments in growth, neurocognitive and psychomotor development, immune function and quality of life.[10–18] For example, chronic anemia has been demonstrated to impair linear growth at all stages of pediatric growth (infancy, childhood and adolescence); however, there is evidence that, in some cases of anemia, adequate iron therapy can result in improved growth and even catch-up growth.[10] The presence of anemia in infancy and early childhood has been associated with impaired neurocognitive development including reduced language skills, short-term memory encoding and socioemotional function, as well as reduced motor function and decreased body balance-coordination skills.[11–16] Unfortunately, there is also evidence that in some cases of IDA in infancy, deficits in cognitive and psychomotor skills can persist even 10 years later despite treatment.[16]

The causes of anemia are varied and include inherited defects in hemoglobin or RBCs (*e.g.* sickle cell disease, thalassemia), infection, chronic diseases or inflammatory disorders (*e.g.*

leukemia, renal disease, inflammatory bowel disease [IBD]), nutritional deficiencies (*e.g.* iron, vitamin B12, folate), acute or chronic blood loss, or medication side effects which can cause increased RBC destruction (hemolysis) or decreased RBC production (myelosuppression).[19] Accurate classification and diagnosis of the underlying cause of anemia is important for effective management. Categorizing anemia by the mean corpuscular volume (MCV), a measure of RBC size, is a common means used to guide an organized approach to investigations to determine the underlying cause of anemia (Figure 1).[19] Microcytic, normocytic and macrocytic anemias are defined by a low, normal and high MCV respectively. Initial classification and diagnosis of the specific causes of anemia is dependent on the assessment of a number of hematologic indices, as well as additional investigations specific to the underlying etiology.

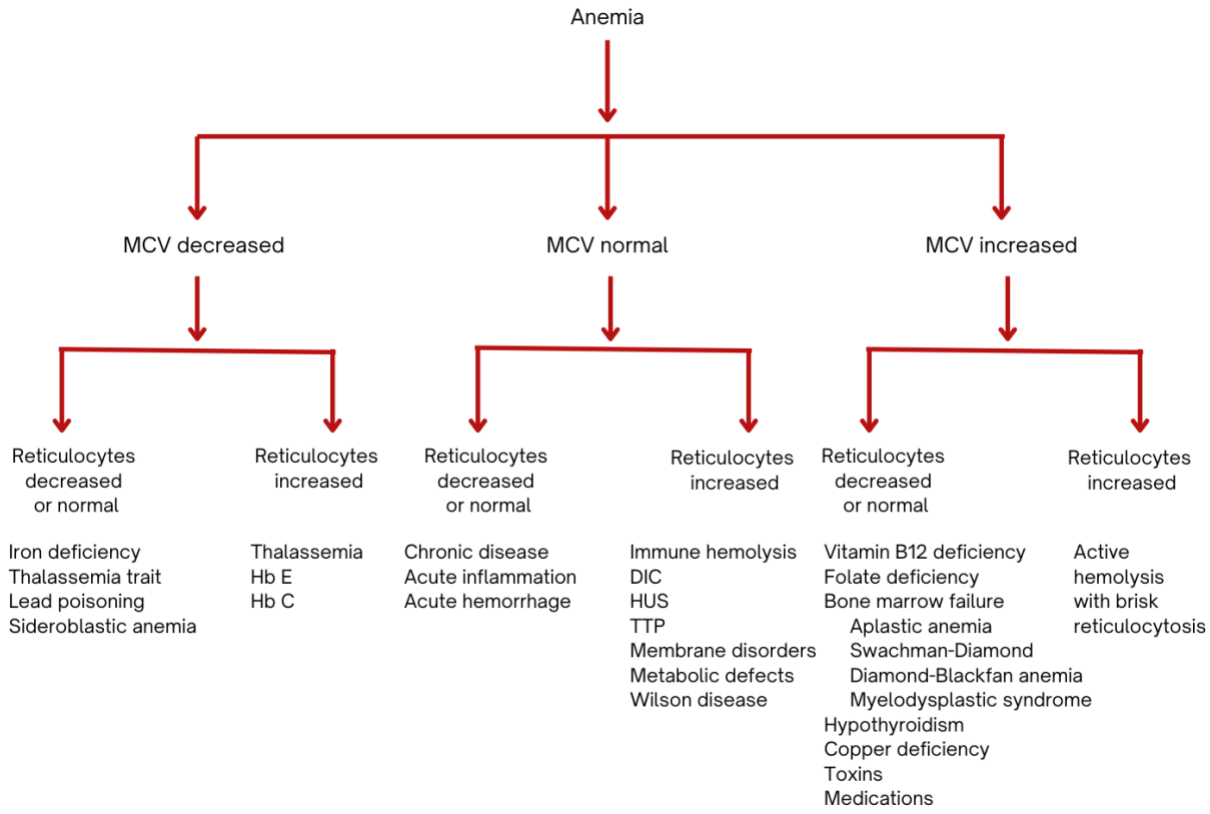

Figure 1. Approach to anemia based on mean corpuscular volume and reticulocyte count.

(adapted from Gallagher, P. [2022]). MCV=mean corpuscular volume; Hb=hemoglobin; DIC=disseminated intravascular coagulation. HUS=hemolytic uremic syndrome. TTP=thrombotic thrombocytopenic purpura.

The global burden of anemia is large, affecting approximately 1/3 of the world population.[20] However, the estimated prevalence of anemia in Canada is < 5% in school-aged children.[21] Iron deficiency anemia (IDA) is the most common cause of anemia and is characterized by a reduction of total body iron stores, also referred to as absolute iron deficiency.[22] Children are at an increased risk of anemia as their increased growth can rapidly deplete iron stores.[23] Anemia of inflammation (AI) is considered the second most prevalent anemia and is due to immune system activation resulting in (i) the inability to use iron stored in the body (functional

iron deficiency), (ii) decreased production of RBCs, (myelosuppression), and (iii) decreased RBC lifespan.[24] [25] In AI, inflammatory signals stimulate the synthesis of hepcidin, a peptide hormone produced by the liver. Hepcidin inhibits the export of iron from cells into the bloodstream by blocking the iron transporter ferroportin 1 (FPN1). The trapped iron is not accessible for new RBC production, thus resulting in a functional iron deficiency anemia, as opposed to an absolute iron deficiency, as seen in IDA. Nutritional anemias are another, less common form of anemia due to deficiencies in vitamin B12 and/or folate which result in ineffective RBC production, also known as erythropoiesis.[26]

1.2.2 Iron Physiology

An understanding of iron physiology and homeostasis is required to understand the role of iron in IDA, AI and mixed IDA/AI. Iron is essential for life. It is required for many processes, including erythropoiesis, oxidative phosphorylation, enzyme activity and DNA synthesis.[27] Circulating iron is utilized by the bone marrow for erythropoiesis and specifically, the synthesis of hemoglobin. At the end of their life span, RBCs are engulfed by macrophages where iron is released and can be effluxed to re-enter the iron pool. The liver is a major storage site for iron and iron can be release or stored in the liver in response to the levels of iron in the iron pool.

Iron absorption is regulated by total body iron requirements and the bioavailability of iron. The duodenum is the main site of absorption of iron from enterally ingested food. Non-heme iron (dietary iron from plants and fortified foods) represents the largest fraction of dietary iron; it is released as ferric iron (Fe^{3+}) and reduced to ferrous iron (Fe^{2+}) by gastric acid and duodenal cytochrome b (DCYTB) (Figure 2). The Fe^{2+} iron is then transported into the cell cytoplasm via

divalent metal transporter 1 (DMT1) located in the apical membrane of the enterocyte. Notably, children with SBS tend to have diets low in fruit and vitamin C is a reducing agent which enhances function of DCYTB.[28,29] Proton-pump inhibitor (PPI) use is also high in children with SBS which reduces gastric acid and thus can inhibit iron absorption. Heme iron (dietary iron from meat) is absorbed directly into the cell by heme carrier protein 1 (HCP1) by receptor mediated endocytosis. Once inside the cell, iron has 3 potential fates; it is utilized by the cell, stored in the cell bound to ferritin, or effluxed out of the cell. This fate of iron is determined by the body supply of iron. If body stores are filled, most iron will be stored within the iron carrier protein ferritin in enterocytes or macrophages. The storage of iron in ferritin is critical as it prevents iron mediated oxidative damage in cells by free iron. If iron stores are low, most of the free intracellular Fe^{2+} will be transported across the basolateral membrane into the plasma via the transport protein FPN1. Once in the plasma, Fe^{2+} is re-oxidized to Fe^{3+} by hepahestin and binds to plasma transferrin and is available for exchange and utilization throughout the body where it can be captured by cells expressing transferrin receptors on their surface. When transferrin saturation (TSAT) (a measure of the amount of iron bound to transferrin) is greater than 75%, free iron spills into serum and can increase susceptibility to gram negative infections, which is of important significance in children with IF and central lines.[30,31] Ferritin and transferrin receptors are both regulated by iron levels. When iron levels are high, transferrin receptor expression is decreased whilst ferritin expression is increased.

Absorption of iron is thought to be regulated at two sites in the apical cell membrane in the duodenum; DCYTB and DMT1. When iron stores are low, synthesis of DCYTB and DMT1 are upregulated to promote increased absorption and transport of iron from the lumen into the blood

for distribution around the body. Iron homeostasis is also tightly regulated by hepcidin, a hormone secreted by the liver. Hepcidin inhibits the export of intracellular iron into the plasma by binding to and inactivating FPN1. As a result, iron becomes trapped within enterocytes and macrophages, which further downregulates iron absorption by inhibiting expression of DCYTB and DMT1.[31,32] Hepcidin synthesis is induced in the liver by inflammatory signals which are mediated by interleukin-6 (IL-6) and bone morphogenetic protein-6 (BMP6) (Figure 2).[33] Hepcidin synthesis is induced by inflammation and suppressed by iron deficiency anemia, hypoxia and oxidative stress. Inflammation triggering increased hepcidin can result in a functional iron deficiency whereby iron becomes trapped intracellularly resulting in insufficient utilizable iron for erythropoiesis despite adequate iron stores. This is one of the mechanisms of AI, as described above, along with reduced iron absorption, inflammatory suppression of erythropoietic activity and decreased erythrocyte survival.[25] Hepcidin is appropriately low in ID, hemorrhage and hemolysis and is high in chronic kidney disease, rheumatologic disease, IBD, infection and iron refractory IDA.[31,32] While there are mechanisms to inhibit iron absorption from the GI tract and prevent iron overload, there is no active excretory mechanism for iron. Thus, parenteral administration of iron can lead to iron overload, which can result in damage to the liver.

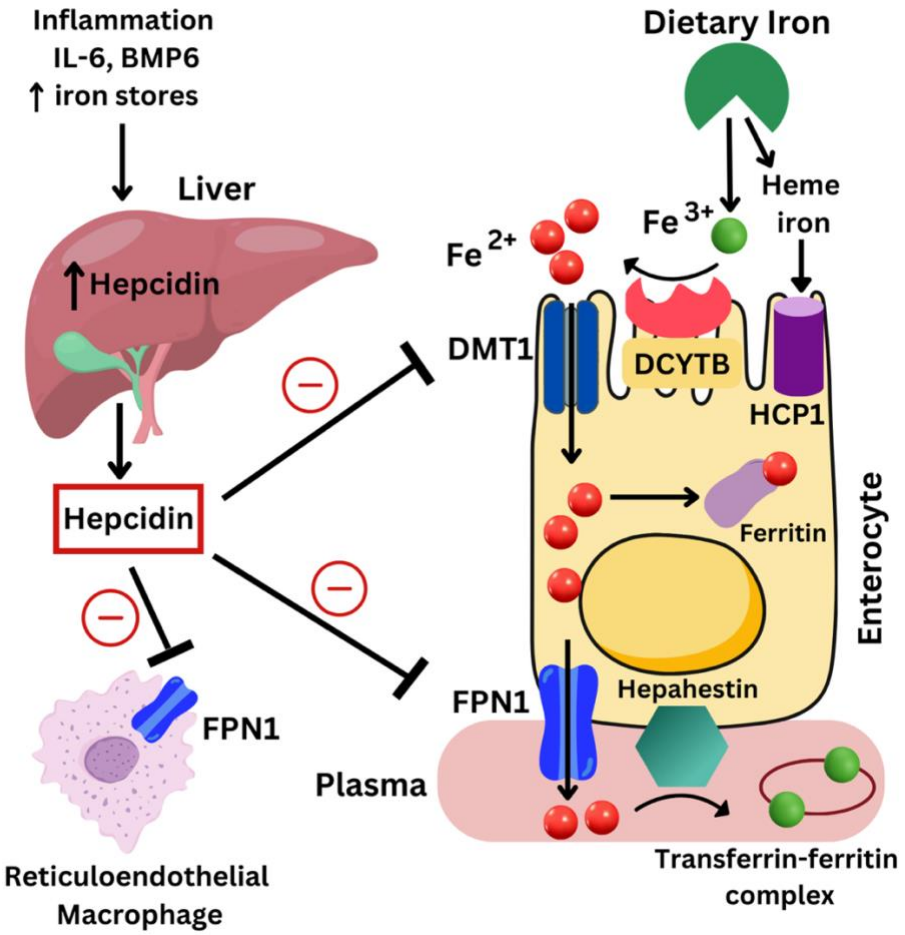

Figure 2. Regulation of absorption and transport of enteral iron.

Hepcidin tightly regulates iron homeostasis. Hepcidin synthesis in the liver is induced by elevated iron stores and inflammation, mediated by interleukin-6 (IL_6) and bone morphogenetic protein 6 (BMP6). Hepcidin has receptors on villus enterocytes, hepatocytes, and reticuloendothelial macrophages. Hepcidin inhibits both iron release and iron absorption by enterocytes in the duodenum. It acts by binding and inactivating ferroportin 1 (FPN1), leading to reduced efflux of cellular iron into the plasma. High intracellular iron inhibits divalent metal transporter (DMT1), further inhibiting iron absorption. Hepcidin also inhibits FPN1 on macrophages, preventing release of ferritin into the bloodstream for circulation and exchange.

1.2.3 Diagnosing IDA, AI and Mixed IDA/AI

Iron deficiency is the first step in the development of IDA and is defined by low iron stores.[34] The gold standard for detection of iron deficiency is the absence of stainable iron on bone marrow aspirate.[35] However, this is impractical for routine use as it is invasive and expensive. Thus, iron deficiency is usually assessed using laboratory markers in peripheral blood. Serum iron is often assessed as part of a panel of "iron studies" and is typically low in IDA. However, serum iron is not a measure of iron stores as it simply reflects the movement of iron in and out of the plasma pool. Serum ferritin is the primary storage form of iron. It has been shown to be a reliable marker of bone marrow stores and is the most effective test to detect iron deficiency.[36] Ferritin < 12-15 μg/L is confirmatory for iron deficiency, however, a cut-off of ferritin < 30 μg/L is more frequently used as it increases the sensitivity to detect iron deficiency from 25% to 92% with a specificity of 98%.[37,38] A major diagnostic limitation of ferritin is that it is also an acute phase reactant which increases in the presence of inflammation and thus, its accuracy in detecting iron deficiency is diminished in the presence of acute or chronic inflammation. TSAT is a measure of the availability of utilizable iron and is another marker of iron status which is readily available. A TSAT < 15% is typically used in screening for iron deficiency but the cut-off is increased to < 20% in the presence of inflammation.[24,33] A threshold of TSAT< 20% has been demonstrated to correlate with iron deficiency detected in bone marrow in chronic inflammatory conditions, including heart failure and chronic kidney disease.[39–42] TSAT alone has a relatively high sensitivity for detecting iron deficiency (up to 90%), but lower specificity (63% to 84%).[39–42] However, one study in patients with kidney disease demonstrated that the specificity improved to 98% when TSAT <20% was combined with ferritin < 100 μg/L.[43] One limitation of TSAT is that serum iron

levels are required for the calculation of TSAT and serum iron levels show diurnal fluctuation and can be influenced by iron in the diet and oral iron supplementation.[44] Thus, current practice for diagnosis of iron deficiency is to use serum ferritin < 30 μg/L in the absence of an inflammatory condition/state and serum ferritin < 100 μg/L or TSAT < 20% in inflammatory conditions. In cases where serum ferritin is 100-300 μg/L, a TSAT < 20% is required to confirm iron deficiency.[24]

AI usually presents as normocytic anemia in the presence of an underlying inflammatory condition, but it may also present as a microcytic anemia, similar to IDA. In addition to low hemoglobin, individuals with IDA or AI typically have low serum iron and TSAT as well. Ferritin is a useful measure to distinguish IDA and AI, as ferritin is low in IDA and elevated (typically > 100 ug/L) in AI.[25] However, IDA and AI can occur together (mixed IDA and AI), and as a result, ferritin may be low, normal or elevated and MCV may be low or normal in mixed IDA/AI. There is no single test which can be used in isolation to classify anemia as IDA, AI, or mixed, and instead, a pattern of numerous key hematologic and biochemical indices, including hemoglobin, MCV, ferritin, total iron binding capacity (TIBC), TSAT, and C-reactive protein (CRP) or other markers of inflammation, is used for classification (Table 1).[24]

Table 1. Pattern of test results used to differentiate iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed IDA/AI.

	IDA	AI	Mixed IDA + AI
Hemoglobin	↓	↓	↓
Mean corpuscular volume (MCV)	↓	↓/normal	↓/normal
Ferritin	↓	↑	↑
Iron (unreliable)	↓	↓	↓
Transferrin saturation (TSAT)	↓	↓/normal	↓
Total iron binding capacity (TIBC)	↑	↓/normal	↓/normal
C-reactive protein (CRP)	Normal	↑	↑
Soluble transferrin receptor (sTfR)	↑	Normal	↑
Solube transferring receptor-ferritin index (sTfR-F index)	↑	↓	↑

Adapted from Weiss et al. (2019) and Goyal et al. (2020).

Additional hematologic markers to determine iron status and differentiate between a functional iron deficiency as seen in AI and absolute iron deficiency in IDA have been available for several years. Soluble transferrin receptor (sTfR) is a test which has shown promise in research settings but is not yet widely available as a routine standard of care test. sTfR is a highly sensitive and specific measure of iron deficiency which is not impacted by inflammation or hepatic disease.[45] Soluble transferrin receptor is a truncated form of the transferrin receptor and is present in every cell. The serum concentration of sTfR is proportional to the total number of transferrin receptors expressed on cell membranes, thus reflecting cellular iron requirements (an

increase in transferrin receptors indicates increased iron needs).[46,47] The sTfR concentration increases in IDA and remains normal in AI. Thus, an increase in sTfR in the presence of AI suggests the presence of concomitant iron deficiency (mixed IDA/AI).[48] The soluble transferrin receptor/log ferritin index (sTfR-F index) may further increase diagnostic accuracy as it is based on the relationship between transferrin (an indicator of iron availability for erythropoiesis) and ferritin (a measure of iron stores). It is calculated by dividing the sTfR in mg/L by the log value of ferritin in μg/L. Both sTfR and the sTfR-F index have been demonstrated to effectively discriminate between IDA, AI and mixed IDA /AI in a variety of populations, including rheumatoid arthritis, pediatric IBD and young children hospitalized or at risk for various chronic infections or inflammatory states.[49–52] The use of ferritin, sTfR and sTfR-F index more than doubled the detection of iron deficiency from 41% (with ferritin alone) to 92% in one study.[53] The ability to distinguish IDA from mixed IDA/AI and AI using sTfR and sTfR-F index would be particularly advantageous in the pediatric IF population, as it may 1) identify the presence of AI in the absence of elevated markers of systemic inflammation, indicating the need for further investigation into potential causes of AI requiring treatment, such as intestinal inflammation or liver or renal disease, and 2) detect iron deficiency before it progresses to IDA, thus triggering further investigations into the cause of iron deficiency, resulting in earlier treatment. This is important as a recent study indicated that parents of children with IF prefer pre-emptive treatment of iron deficiency as opposed to a reactionary approach to IDA which may result in the need for invasive iv iron therapies or blood transfusions for management.[54]

1.3 Anemia in Pediatric Intestinal Failure

1.3.1 Prevalence and Classification

Anemia is a frequent complication in children with IF with the reported prevalence ranging from 40% in a single-center cross-sectional study to as high as 90 to 97% in three, single-center, retrospective studies.[55–58] The higher prevalence in the retrospective studies reflects the ability to assess multiple blood samples over several years as opposed to a single blood sample in the cross-sectional study. As the primary aim of each of these studies was to assess micronutrient deficiencies rather than anemia, the underlying causes of anemia were not evaluated.

Children with IF are at risk for both IDA and AI however data is limited on the epidemiology of IDA and AI in children with IF. To date, only one study has evaluated the prevalence of IDA and AI in the IF population.[59] This single center, retrospective study included 54 adult and pediatric IF patients who had been on PN for more than 5 years. Using serum levels of ferritin with TSAT, the prevalence of AI was 36%, IDA 38% and anemia with indeterminate iron status was 21%. The authors evaluated possible causes of AI, specifically renal and liver disease, and demonstrated that 23% of patients met criteria for chronic kidney disease (CKD) stage 2 or 3. However, the retrospective study design was a considerable limitation of the study, as the necessary investigations to thoroughly assess the potential causes of AI, such as intestinal failure associated liver disease (IFALD), renal disease and intestinal inflammation, as well as the underlying causes of IDA, were absent, thus highlighting an important gap in the research.

Differentiating between IDA, AI and mixed IDA/AI is critical to ensuring appropriate identification and treatment of the underlying cause of anemia. Iron is a micronutrient which is essential to the survival of nearly all living cells, including microbes.[60] The sequestration of iron

seen in AI is believed to be an evolutionary strategy termed “nutritional immunity” to make iron less accessible to circulating pathogens, therefore inhibiting the ability of potential pathogens to survive, replicate and invade host cells.[61,62] Indeed, conditions associated with iron overload, such as hereditary hemochromatosis and thalassemia are associated with increased risk of infection.[63] Indiscriminate iron supplementation can lead to increased morbidity and mortality, including enteric infections due to bacterial utilization of excess iron, which is of particular concern in the pediatric IF population, as these children are at high risk for enteric infections and sepsis, specifically in the presence of a central venous catheter for PN.[25] Iron overload from aggressive iron supplementation can also contribute to liver fibrosis.[64] On the other hand, iron deficiency in infancy and childhood is associated with negative effects on growth, immune function, intestinal adaptation, motor and neurocognitive development, and school achievement, and therefore, failure to identify and treat concomitant iron deficiency in a child with AI may contribute to long-term morbidity.[10,11,17,18] An improved understanding of the types and underlying causes of anemia in pediatric IF is essential to ensure effective management and improve long-term outcomes.

1.3.2 Potential Etiologies of Iron Deficiency Anemia in Pediatric Intestinal Failure

The potential underlying causes of IDA in children with IF are varied and can be broadly classified as inadequate iron intake, inadequate iron absorption and increased gastrointestinal losses.

1.3.2.1 Inadequate Iron Intake

Iron is an essential micronutrient obtained through the diet. There are 2 types of iron; heme iron, which is found in animal meat (e.g. beef, chicken, fish) and non-heme iron, which is found in plant products (e.g. spinach, lentils, tofu). Infants and children require 7-15 mg per day of iron from their diet depending on their age and sex.[23,65] Children who have limited enteral intake due to oral aversion, feed intolerance or malabsorption secondary to IF are dependent upon iron supplementation in order to meet their iron requirements. Iron supplementation can be achieved by oral/enteral administration of iron fortified formulas/cereals or elemental iron supplements or via intravenous (IV) iron infusions. In cases of severe IDA, blood transfusions are required. Adherence to oral/enteral supplementation is frequently low in children due to oral aversion, poor palatability, or gastrointestinal side effects such as abdominal pain and diarrhea and concerns regarding anaphylaxis and incompatibility prevents direct supplementation of iron in PN mixtures in many centres, including those that manage pediatric IF in Western Canada.[54,66,67] Thus, achieving adequate iron supplementation can be challenging. Persistent IDA in the presence of adequate supplementation and adherence is an indication for further investigations into the underlying cause, including intestinal inflammation or occult GI bleeding.

1.3.2.2 Inadequate Iron Absorption

Mucosal inflammation in the small bowel inhibits the absorption of Iron and associated nutrients which are absorbed in the duodenum and proximal jejunum.[68] Evidence of acute and/or chronic intestinal inflammation diagnosed by endoscopy/colonoscopy has been reported in several studies in children with IF.[69–72] Endoscopy is not routinely performed without specific

clinical indications, such as worsening malabsorption or frank GI bleeding and thus, there is a paucity of information regarding the true prevalence of intestinal inflammation in pediatric IF. A single retrospective study describing surveillance endoscopy found histological intestinal inflammation in 54% of "asymptomatic" children with IF.[71] Unfortunately, the underlying cause for this inflammation was unknown and anemia was not assessed. Potential contributors to small bowel inflammation and IDA include small intestinal bacterial overgrowth (SIBO) and eosinophilic/allergy related inflammation.[73–76]

SIBO is characterized by qualitative and quantitative changes in endogenous bacteria in the small bowel and is estimated to occur in 34-75% of pediatric SBS patients.[74,77] Children with IF are at increased risk of SIBO due to alterations in intestinal motility and anatomy, including loss of the ileocecal valve (ICV) and small bowel dilatation due to intestinal adaptation, as well as increased exposure to antibiotics and gastric acid suppressing medications.[78] SIBO contributes to IDA through intestinal inflammation and mucosal injury, as evidenced by endoscopic ulcerations and villous blunting on histology, resulting in impaired absorption of micro and macronutrients, including iron. In addition, SIBO may cause a macrocytic anemia secondary to bacterial utilization of vitamin B12.[79] An accurate and non-invasive diagnostic test for SIBO in pediatric IF does not currently exist. Hydrogen breath tests are frequently used to diagnose SIBO, however, their accuracy in children with altered intestinal anatomy and function is not known and thus, in the pediatric IF population, SIBO is a clinical diagnosis based on increased stool outputs, abdominal distention, gas or D-lactic acidosis which are not otherwise explained and which improve with empiric antibiotic treatment.[77,80] Interestingly, fecal calprotectin (FCP), a stool marker specific for intestinal inflammation, was previously found to be more elevated in children with SBS with

confirmed SIBO compared to children with SBS without SIBO.[74] The potential role of FCP as a non-invasive marker of SIBO or intestinal inflammation in general has not been thoroughly evaluated in the pediatric IF population and may be useful in identifying the causes of anemia in pediatric IF.

Eosinophilic gastroenteritis is an inflammatory condition which can affect the upper and/or lower GI tract and is characterized by infiltration of the tissue by eosinophils, a type of white blood cell frequently associated with allergic conditions.[75] Following several case reports of eosinophilic inflammation in IF, a large, retrospective study of children with IF who had undergone endoscopy and/or colonoscopy reported an overall prevalence of eosinophilic intestinal inflammation diagnosed by histology of 37%.[69,70,81] While the presence of anemia was not evaluated in this cohort, anemia has been found to be associated with eosinophilic gastroenteritis in otherwise healthy children and thus, may be an important contributor to anemia in pediatric IF.[76]

1.3.2.3 Gastrointestinal blood loss

Anastomotic ulceration (AU) is a known cause of anemia in short bowel syndrome.[82,83] The etiology of AU is unknown, but ischemia, SIBO, excessive acid, bile salt exposure and allergy have been proposed.[82] AU may require surgical intervention or treatment with anti-inflammatory medications such as steroids, sulfasalazine or infliximab.[82,84] AU is associated with occult GI blood loss and thus, diagnosis of AU may be delayed several years, resulting in prolonged oral or intravenous iron supplementation for IDA while failing to identify the underlying cause.[84] In the course of evaluation and management of chronic anemia in the IF population at Alberta Children's

Hospital, we have detected a high incidence of intestinal ulceration; ulcers have been identified in 10 of 32 patients (31%) in our current cohort (unpublished data). This is strikingly higher than the reported 30-year AU prevalence of 0.3% in the pediatric IF population in France.[82] Interestingly, ulcers in our patients occur not only at anastomosis sites and staple lines from bowel lengthening procedures, but also at other sites within the GI tract. The occurrence of non-anastomotic ulceration has not been described in detail the pediatric IF literature and thus, characterization of the prevalence of GI ulcers may help to elucidate their role in anemia and indicate a need for routine endoscopy in evaluation of anemia.

1.3.3 Potential Etiologies of Anemia of Inflammation in Pediatric Intestinal Failure

With only 1 study to date examining the prevalence of AI in pediatric IF, the underlying causes remain largely unknown.[59] Potential contributors include renal disease, liver disease and chronic intestinal inflammation. Anemia is a well described complication of chronic kidney disease (CKD) with multiple etiologies, including inflammation and a decrease in erythropoietin (EPO), a renal hormone which stimulates erythropoiesis.[85] Glomerular filtration rate (GFR) is a measure of renal function and is used in diagnosis and disease staging, with disease progression defined by decreasing GFR. There is growing evidence of renal dysfunction and development of CKD in children with IF.[85–87] Studies in both adults and children on long-term PN have demonstrated a gradual decline in GFR and abnormalities on renal ultrasound, including increased echogenicity and nephrocalcinosis. Increased echogenicity is a finding which can be due to inflammation, tubular disorders or fibrosis, while nephrocalcinosis is caused by precipitation of minerals within the renal parenchyma. The causes of CKD in long-term PN

remains largely unexplained.[88] However, recurrent episodes of sepsis, dehydration, electrolyte imbalances, nephrotoxic medications and impaired intestinal absorption are proposed mechanisms which exist in IF patients.[87] A retrospective study of 54 pediatric IF patients found sonographic renal abnormalities in 41% of patients while another study reported a decrease in GFR in 29%.[85,86] In both studies, increased PN duration and shorter remaining bowel length were associated with impaired renal function. Anemia was not assessed in these studies, however, Namjoshi et al. (2020) found evidence of CKD in 23% of their patient cohort, which suggests that CKD may be an important contributor to AI in this population.

Intestinal failure associated liver disease (IFALD) is a major source of morbidity and mortality in pediatric IF. IFALD is characterized by a persistent elevation in bilirubin and eventual progression to cirrhosis and liver failure.[89] The role of chronic liver disease in AI is poorly understood, however, Namjoshi et al. (2020) did find an association between elevated bilirubin and AI, suggesting a potential role for IFALD in AI in children with IF. This association requires further investigation, including correlation with ultrasound findings.

Chronic intestinal mucosal inflammation is a potential contributor to AI in children with IF which requires further investigation. There is a growing number of case reports describing the development of chronic inflammation resembling IBD in children with IF and post-surgical resection of the bowel, as well improvement in inflammation after treatment with medications routinely used in IBD treatment, such as budesonide, 5-aminosalicylates (5-ASA) and biologics.[69,71,90–94] Anemia is a frequent and persistent complication in children with IBD and while the precise etiology of IBD is still unclear, there is evidence that it results from the interaction of dysbiosis in the intestinal microbiota and resulting impaired mucosal defense activities along with

an altered immune response in genetically predisposed indviduals.[33] Shifts in the composition of the gut microbiome can lead to impaired intestinal epithelial barrier function and result in immune activation and cytokine production triggering local and systemic inflammation.[95,96] Alterations in the gut microbiome of children with SBS have been reported in several studies, demonstrating reduced microbial diversity akin to that seen in IBD and thus, it is reasonable to hypothesize that this dysbiosis contributes to intestinal inflammation in children with SBS/IF.[97,98] Furthermore, dysbiosis has been associated with poor growth, need for longer PN duration and IFALD in children with IF. [97,98] In addition, this reduced intestinal microbial diversity can further predispose children with IF to SIBO, which has also been found to be associated with evidence of intestinal inflammation in children with IF.[74,99] Persistent intestinal inflammation has also been reported in children with SBS at initial and subsequent serial transverse enteroplasty (STEP) procedures.[72] STEP is a surgical technique to reduce abnormal small bowel dilation that occurs as an adaptive response to reduced bowl length in some children with SBS. This bowel dilation predisposes to SIBO and provides further support for the role of dysbiosis and SIBO in intestinal inflammation in children with IF.[100] Interestingly, intestinal dysbiosis has also been found to be associated with IFALD in children with IF and thus, the altered gut microbiota in children with IF could contribute to AI through multiple mechanisms.[101]

1.3.4 Nutritional Anemia and the Role of Micronutrient and Vitamin Deficiencies in Anemia in Pediatric Intestinal Failure

Nutrients are absorbed differentially throughout the GI tract and therefore, the resultant length and anatomy of the GI tract following intestinal resection in SBS will predispose to specific

micronutrient and vitamin deficiencies.[102] For example, children with resection of the duodenum and proximal jejunum are at risk for iron deficiency while resection of the ileum is a risk for vitamin B12 deficiency and fat soluble vitamin deficiency (vitamins A, D, E and K) secondary to bile malabsorption.[6] Micronutrients and vitamins play a critical role in GI structure and function and deficiencies may inhibit the intestinal adaptation necessary to achieve enteral autonomy.[4] Several small cross-sectional studies ($n \leq 10$) and larger single center, retrospective studies ranging in size from 30 to 178 study participants have reported a high prevalence of micronutrient and vitamin deficiencies in children with IF on PN and after transition to full enteral nutrition (EN).[55–57,103–105] Using regression analysis, Ubesie et al, (2013) and Namjoshi et al, (2018) found that longer PN duration was associated with micronutrient deficiencies, while Yang et al (2011) found the presence of the ICV was protective for micronutrient deficiencies. The prevalence of deficiencies was particularly high for zinc (20-52%), copper (29-56%) and vitamin D (20-38%).[55–57] These vitamins and micronutrients are of particular relevance in anemia as zinc and copper have roles in iron metabolism and hemoglobin synthesis and have been found to perpetuate IDA, while there is growing evidence of a relationship between vitamin D deficiency and AI.[57,106–108]

1.3.4.1 Role of Zinc in Anemia

Zinc is an essential micronutrient which serves as a cofactor in several enzymes and metabolic processes including iron metabolism and erythropoiesis and thus, plays as role in iron-deficiency and anemia.[109, 110] Zinc is absorbed mainly in the duodenum and small intestine and has been demonstrated to be a strong predictor of hemoglobin concentration in healthy school age children and adults.[111,112] There is no storage form of zinc in the human body and thus, zinc

status is determined by daily oral intake/absorption of zinc-containing foods (chicken, meat, lentils, nuts) and/or supplementation of PN.[113] Zinc may also contribute to AI as deficiency has been demonstrated to induce pro-inflammatory cytokines and aggravate mouse models of colitis and has also been found to correlate with increased serum levels of pro-inflammatory markers including IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), CRP and FCP in patients with rheumatoid arthritis and IBD.[114–117] Plasma zinc is a reliable means of assessing zinc status, however, albumin is the main carrier protein of zinc and zinc is also a negative acute phase reactant and thus, zinc deficiency must be interpreted with caution in the presence of hypoalbuminemia and inflammation.[118]

1.3.4.2 Role of Copper in Anemia

Similar to zinc, copper serves as a cofactor for a variety of important enzymes and metabolic processes including hemoglobin synthesis and iron oxidation.[119] Deficiency can present with anemia and neutropenia.[120] Low levels of copper can result from inadequate dietary intake or PN supplementation, inadequate absorption in the GI tract, which occurs mainly in the stomach and proximal duodenum but also in the jejunum, or increased losses due to malabsorption and diarrhea.[121] Copper shares some common transporters with both zinc and iron and thus, these micronutrients may compete for absorption.[122] Excess zinc supplementation can cause a copper deficiency anemia which may be microcytic, normocytic or macrocytic. Copper deficiency can be detected by serum copper levels and copper increases in response to inflammation. Ceruloplasmin, a major copper-carrying protein in the blood which also plays a role in iron metabolism will be low in serum only in the presence of severe copper deficiency.[123]

1.3.4.3 Role of Vitamin D in Anemia

An association between Vitamin D deficiency and AI has been described in various populations including children with CKD and IBD.[124,125] *In vitro* studies have demonstrated the role of vitamin D in decreasing hepcidin induced pro-inflammatory cytokines levels via downregulation of hepcidin expression, and vitamin D supplementation has resulted in decreased serum hepcidin levels and decreased need for erythropoietin stimulating agents in patients with CKD.[126–130]

1.3.4.4 Role of Vitamin B12 and Folate in Anemia

Vitamin B12 and folate both play critical roles in DNA and RNA synthesis and deficiencies in either of these vitamins result in ineffective erythropoiesis characterized by production of fewer, but larger abnormal red blood cells and premature cell death.[26] Nutritional anemias due to vitamin B12 and/or folate deficiency are macrocytic and are associated with anisocytosis, poikilocytosis and hypersegmented neutrophils on blood smear.[131] Vitamin B12 is found in animal products (meat, eggs, dairy) while folate can be obtained from ingestion of vegetables, especially leafy greens as well fortified flours and cereals.[132] Vitamin B12 requires adequate gastric acid to release it from dietary proteins and it is absorbed in the terminal ileum. Children with IF are at risk for vitamin B12 deficiency if they have short bowel involving surgical resection of >15 cm of the ileum, chronic gastric acid suppression (*e.g.* PPI therapy), or SIBO, as enteric bacteria compete with the host bowel for vitamin B12.[133,134] The risk of folate deficiency is considered low in children with SBS and those at risk for SIBO, as folate is synthesized by enteric flora.[135] Indeed, several retrospective and small case studies have assessed vitamin B12 and folate status in

children with IF and folate deficiency was not present in any of the children, while vitamin B12 was found to be deficient in 6.9% to 22% of patients.[55,56] Vitamin B12 and folate status are evaluated via serum levels, however, deficiencies in associated vitamin B12 binding proteins can result in normal vitamin B12 levels in individuals with functional vitamin B12 deficiencies.[136,137] Therefore, simultaneous assessment of vitamin B12 and methylmalonic acid (MMA), a metabolic product which requires vitamin B12 for metabolism, is recommended.[138] Levels of MMA are elevated in >98% of patients with a clinical vitamin B12 deficiency and is considered to be a highly sensitive and specific test diagnosing vitamin B12 deficiency and levels of MMA decrease with vitamin B12 supplementation.[131,138–140]

Patterns of micronutrient and vitamin deficiencies may help to identify the underlying causes of anemia. For example, vitamin B12 deficiency with elevated MMA and normal folate is observed in SIBO, while deficiencies in fat soluble vitamins (A, D and E) and elevated bilirubin are indications of liver disease.[141,142] Thus, evaluating the associations of micronutrient and vitamin deficiencies with AI and IDA may identify predictors useful in diagnosis and monitoring of anemia in pediatric IF.

Many micronutrients and vitamins are acute phase reactants and should be interpreted cautiously in the presence of a systemic inflammatory response. Vitamins A, D, E and B12, as well as zinc all decrease in the presence of inflammation, while copper increases.[118] A systematic review evaluating the magnitude of changes of serum vitamin and micronutrient levels in the presence of a systemic inflammatory response, as measured using CRP, found that the levels of zinc, copper and vitamins A, D, E and B12 were within the normal reference range in the presence of mild inflammation (CRP < 10 mg/L).[118] In the presence of major inflammation (CRP >80 mg/L)

the concentrations of vitamin B12, vitamin E and copper changed by approximately 10%, while the concentrations of zinc and vitamins A and D decreased by 25 to 55%. Correction of plasma micronutrients using CRP and albumin have been examined for zinc, vitamin A and vitamin D, and may be a means to assess micronutrient/vitamin status more accurately in patients with chronic inflammation.[143] [144]

1.3.5 Medications and Additional Causes of Anemia

Medications are an important cause of anemia in all children and should not be overlooked in children with IF. Medications can contribute to anemia through various mechanisms, including hemolysis (*e.g.* septra), myelosuppression (*e.g.* 5-ASA, anticonvulsants, immunomodulators) or inhibition of absorption of vitamins and micronutrients (*e.g.* proton-pump-inhibitors, cholestyramine).[145] Some of these classes of medications are used frequently in children with IF to manage IF specific complications, such as the use of PPIs to manage gastric acid hypersecretion or broad-spectrum antibiotics for SIBO, while other classes of medications may be used to manage other comorbidities, such as anticonvulsants for epilepsy. Therefore, a detailed inventory of patient medications is necessary when evaluating causes of anemia. Similarly, it is also important to assess for additional causes of anemia not specific to pediatric IF, such as thalassemia, hemoglobinopathies or thyroid disorders.[19] These disorders are generally easily identified through additional blood tests once anemia has been diagnosed.[19]

2 Chapter 2: Research Rationale and Objectives

2.1 Research Rationale

Outcomes in children with IF have improved considerably over the last decade and the majority of children are now surviving through childhood.[5] With improved survival, new burdens and complications are becoming more apparent. Anemia is an important complication in the pediatric IF population with a reported prevalence greater than 90%.[55–57,59] Anemia has considerable negative long-term consequences including impairments in growth and neurocognitive development.[10,11] Children with IF are at high risk for IDA, AI and mixed IDA/AI. There is a paucity of data on the frequency of the varied types of anemia in pediatric IF and the underlying etiologies are not well described. An improved understanding of the prevalence and associated contributors of these anemia types is needed to effectively treat and prevent anemia and its complications in children with IF.

2.2 Aims and Objectives

2.2.1 Aims

To describe the frequency of the various types of anemia in the pediatric IF population and identify factors associated with these anemias.

2.2.2 Objectives

Primary Objective: To determine the 10-year period prevalence of anemia in children with IF.

Secondary Objective: To determine the 10-year period prevalence of anemia types of IDA, AI, mixed IDA/AI and nutritional anemia in children with IF.

Exploratory Objectives: There are many potential clinical contributors to the various types of anemia in children with IF. Therefore, we identified a number of exploratory objectives to help identify potential clinical factors and relationships to help generate hypotheses which may guide future research. Potential factors associated with the various types of anemia to be evaluated include underlying IF etiology, surgical history and resulting GI anatomy, micronutrient deficiencies, mechanism/types of iron-supplementation and IF-associated complications, including liver disease, renal disease, small intestinal bacterial overgrowth, evidence of intestinal inflammation as measured by fecal calprotectin, and endoscopic and histologic findings. In conducting this study, the use of iron-supplemented PN was identified as a distinct clinical practice difference between the intestinal rehabilitation programs (IRPs) in Toronto vs Alberta; Toronto uses iron-supplemented PN in nearly all hospitalized and home PN children with IF while iron-supplemented PN is not available in Alberta. This difference has potential effects on the overall prevalence of anemia, as well as anemia types and IF-associated complications and thus, a post-hoc sub-analysis comparing prevalence of anemia, anemia types and IF-associated complications was undertaken to help assess the clinical implications of this difference in practice.

3 Chapter 3: Methods

3.1 Study Design

This was a multicenter, retrospective study of pediatric IF patients managed by three separate IRPs in Canada during the enrolment period (November 1, 2021 – Dec 31, 2023). The IRPs include the Alberta Children's Hospital Children's Intestinal Rehabilitation Program (CHIRP) (Calgary, AB), the Stollery Children's Hospital CHIRP (Edmonton, AB), and the Group for Improvement of Intestinal Function Treatment (GIFT) at the Hospital for Sick Children (Toronto, ON). Together, these hospitals serve a catchment area covering Alberta, Saskatchewan, Northwest Territories, and most of Ontario which includes an estimated total population size of 21.8 million people based on provincial population data from 2023;[146] all children with IF living in these areas are cared for and managed by these IRPs. The pediatric IF population is a small and heterogeneous population; there are <50 children actively followed by each CHIRP team in Alberta, and thus, a multicenter design was necessary to increase sample size and overall study power. The GIFT program in Toronto, ON serves a larger population than the CHIRP teams in Alberta and had previously established a database for their patient population (which included approximately 150 children) for clinical and research purposes. The retrospective chart review encompassed a 10-year observation time span dating back ten consecutive years from the date of enrolment for each participant. The total number of years of observation varied for each participant based on the participant's age at enrolment and date of diagnosis of IF. For example, a child who was 2 years old at enrolment and was diagnosed with IF at birth would have 2 years

of observation, while any child ≥ 10-years of age at enrolment diagnosed with IF at birth would have 10 years of observation.

All children 6 months to 18 years of age with IF, defined as the need for PN for >60 days due to intestinal disease or dysfunction, managed by the IRPs at the study sites were eligible for inclusion in the study. Children did not have to be on PN at the time of enrolment but were required to have been on PN at some point within the 2 years prior to the time of enrolment. The stipulation for PN use within the previous 2 years was included as it was determined during the study design development that there were differences between centers with respect to duration of observation for children with IF after successful weaning off PN. Children who continued to have complications, such as anemia or feeding difficulties, were followed disproportionately in comparison to those without persistent complications and thus, we risked introducing selection bias in our estimates of prevalence of anemia in the pediatric IF population if we included children who were off PN for > 2 years. Written or telephone informed consent and/or assent where appropriate, was required for all participants and participants were required to have a minimum of one complete blood count (CBC) measurement. Children with liver or intestinal transplant were still included if they met the other inclusion criteria, however, retrospective data was only collected up until the time of their transplant as potential risk factors and causes of anemia are different in children following organ transplantation and while on transplant-related immune-suppressing medications.

The study protocol was approved by the research ethics boards (REBs) at each participating site (University of Calgary [REB21-0782], University of Alberta [REB21-0782 psite 00000032], and SickKids Hospital [REB 1000079556]). Children and their legal guardians provided

informed assent and consent for enrolment. Participants followed by the GIFT team in Toronto had previously provided consent for their inclusion in the established local database in Toronto and thus, an additional consent for this study was waived by the REB.

All eligible participants followed by the CHIRP team in Calgary (n=23) and the GIFT team in Toronto (n=54) were consented and enrolled in the study. In Edmonton, 13 of 15 eligible participants followed by the CHIRP team gave consent and were enrolled in the study. The study sample therefore approaches inclusion of nearly the entire eligible IF population followed by these three IRPs.

This observational study is reported in accordance with the Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) statement (Appendix, Table 1-1).[147]

Funding for this study was supported by a Resident Research Award from the Canadian Association of Gastroenterology and by a small research grant from the Alberta Children's Hospital Research Institute.

3.2 Data Collection

Data was retrieved longitudinally from electronic medical records (EMRs) in Alberta and Toronto, as well as from the IF patient database in Toronto. An electronic data abstraction tool created specifically for this study was used for data collection. Data abstraction was performed by the MSc candidate. A medical student also volunteered hours to collect data from the EMRs of participants in Alberta under the supervision of the MSc candidate. Each participant was given a unique participant subject code for use on the data abstraction forms to protect confidentiality. The master list linking the participant subject code and their identifying information was kept in

a locked file cabinet and an electronic copy maintained in a password protected file on a secure University of Calgary server. At the time of enrolment, medical records for all newly enrolled participants followed by the CHIRP teams in Alberta were reviewed for completeness of annual investigations; any missing investigations were requested to be ordered by the primary IF physician for completion of investigations, including those which are not considered routine standard of care, specifically fecal calprotectin and urinalysis. Routine endoscopy is not standard of care at the intestinal rehabilitation centers in this study and is typically only performed for investigations related to GI bleeding, persistent and treatment refractory anemia or unexplained poor growth, malnutrition or GI symptoms. While having at least one endoscopy for each participant would have been useful for completion of data, this study was performed during the coronavirus disease 2019 (COVID-19) pandemic and performing elective endoscopy was not feasible and thus, only retrospective endoscopic data was collected. Requesting additional laboratory or imaging investigations for children followed by the GIFT program was not feasible given that many children are managed remotely by local care teams under the direction of the GIFT team and requesting additional investigations would require involving additional care providers.

Data collected from chart review included: (i) demographic information including date of birth, gestational age, sex, primary etiology of IF and date of diagnosis of IF; (ii) surgical history including dates of surgical procedures, resulting intestinal anatomy and bowel length at last surgery and predicted small bowel length, history of STEP or tapering procedure, dates of gastrostomy (GT) or gastro-jejunal (GJ) tube insertion/removal, dates and histology of liver biopsy; (iii) nutritional information including dates of PN initiation/weaning, use/dates of

Omegavan for PN associated cholestasis/IFALD, modes of enteral feeding (oral, nasogastric/nasojejunal, GT/GJ tube), dietary restrictions (vegan, lacto-ovo vegetarian, low carbohydrate for management of lactic acidosis, nothing by mouth for bowel rest), presence of iron supplementation in PN, oral/enteral/intravenous vitamin or micronutrient supplementation (specifically vitamin D, vitamin B12, folate, iron, copper, zinc); (iv) evidence of intestinal failure associated liver disease (based on liver biopsy histology, ultrasound with dopplers and elastography) or renal disease (ultrasound, urine studies, estimated GFR [eGFR]); (v) history and management of SIBO as determined by clinical symptoms reported in the medical record, dates and use of SIBO antibiotics, positive diagnosis of SIBO from duodenal cultures and histology collected from intestinal mucosal biopsies consistent with SIBO; (vi) history of endoscopy and histologic inflammation or endoscopic abnormalities (mucosal erosions, ulcers, edema, erythema, anastomotic ulcers, staple line ulcers); (vii) medications used to manage IF related complications including PPIs, corticosteroids, budesonide, 5-ASA, biologics, ursodiol, antibiotics for SIBO prophylaxis or treatment or for concomitant conditions (NSAIDs, anti-epileptics); (viii) history of presentation with GI bleeding (hematemesis, hematochezia, melena) and management including endoscopy, packed red blood cell (PRBC) transfusions, definitive or temporizing surgical management/interventions; (ix) growth parameters and clinical symptoms/assessment at time of annual CBC and iron studies including height, weight, clinical symptoms of SIBO and signs of liver disease and portal hypertension on exam, such as hepatomegaly and splenomegaly; (x) comorbid diagnoses relevant to anemia, specifically thalassemia, IBD, seizure disorders, thyroid disease, rheumatologic disorders, cardiac disease, renal disease, cancer and immune disorders.

Annual laboratory (serum, urine, stool) and imaging investigations collected to assess for anemia and evidence of liver disease, renal disease, vitamin/micronutrient deficiencies and systemic inflammation are summarized in Table 2. The frequency of these investigations varies for each patient depending on their age, duration and stability of PN use/prescription and comorbidities/complications. The general protocol for the CHIRP and GIFT teams is to perform annual vitamin and micronutrient testing for children on stable home PN (these labs may be divided into batches performed every 6 months to avoid exceeding blood draw limits at a single blood draw). CBC (+/- iron studies), liver studies and renal studies may be performed at intervals ranging from weekly to every 3 months to once per year depending on the child's status and ultrasound surveillance of liver and kidneys is performed annually after the first year of life. In order to assess for prevalence of anemia in our study, we recorded a single CBC from each calendar year for up to 10 years retrospectively where available; these were referred to as hemoglobin measurement timepoints. Thus, children with only 1 year of observation and a single annual CBC measurement had only 1 hemoglobin measurement timepoint, while a child with 10 years of observation and thus 10 annual CBC measurements had 10 hemoglobin measurement timepoints. In several instances, there was missing data with no CBC measurements in one or more calendar years interspersed between the consecutive years of retrospective data; there were 22 patients from Toronto who had a total of 32 missing annual CBC measurements; no participants in Alberta had missing CBC measurements. All of the participants with missing CBC measurements had anemia on at least one annual CBC measurement, and thus there was no risk of misclassification bias with respect to determination of the period prevalence of anemia. All CBC tests performed within each calendar year were reviewed and the CBC with the lowest

hemoglobin along with accompanying iron studies and inflammatory markers (CRP or erythrocyte sedimentation rate [ESR]) was collected. If there was only a single CBC where the hemoglobin was anemic for age during the year, but it had no accompanying iron or inflammatory studies, we recorded that hemoglobin in order to document the presence of anemia, knowing that we would not be able to classify the anemia type. Where possible, we recorded labs performed at least 6 months apart between calendar years in an effort to avoid documenting labs from the same episode of anemia without allowing time for the anemia to be treated. Any labs, specifically CBC, iron studies and inflammatory markers performed within 4 weeks of a major surgery or acute illness or infection including central line associated blood stream infections/sepsis, urinary tract infection, respiratory illness confirmed by molecular testing (e.g. polymerase chain reaction studies positive for influenza, COVID or entero-rhinovirus), or infectious gastrointestinal illness confirmed by culture or molecular methods (e.g. norovirus, *clostridium difficile*) were not included, as these events could serve as causes and/or confounders to anemia unrelated to IF status. Vitamin and micronutrient levels collected in the presence of evidence of acute systemic inflammation with CRP > 10 mg/L were not utilized in analysis to ensure accurate interpretation and to limit classification bias given that there is evidence that levels can be falsely elevated or depressed in the presence of inflammation.[118,143,144]

Table 2. Investigations for evaluation of the causes of anemia and potential predictors

Category of Investigation	Specific Investigations	Rationale/utility for Investigations
Hematologic	• CBC + differential, MCV, MCHC, RDW, reticulocyte count • Iron, ferritin, Tsat, TIBC, sTfR	• Identification and classification of anemia
Micronutrients and Vitamins	• Vitamin A, D, E, B12; Folate, MMA • Copper, ceruloplasmin, zinc	• Contributors to anemia • Nutritional anemia
Inflammation	• Albumin, WBC, platelets • CRP, ESR, Fecal calprotectin	• Identify evidence of inflammation for AI
Liver and Renal Disease	• ALT, AST, ALP, GGT, bilirubin, INR, albumin • Creatinine (for eGFR), urinalysis • Renal ultrasound • Liver ultrasound with elastography	Evidence of intestinal failure associated liver and renal disease
Endoscopy and Histology	• Upper endoscopy and/or colonoscopy with histology • Capsule endoscopy	• Identify presence of intestinal ulcers/bleeding • Histologic evidence of intestinal inflammation
Additional investigations to identify underlying causes of anemia not specifically related to IF	• Blood smear – hemoglobinopathies, lead poisoning, hemolysis • G6PD screening test • Lead level – lead poisoning • Haptoglobin, LDH, indirect bilirubin, DAT – hemolytic anemia • Hemoglobin electrophoresis - thalassemia • Osmotic fragility – hemoglobinopathies • Thyroid hormones – thyroid disease	• Rule out other causes of anemia – only ordered as deemed necessary

CBC=complete blood count; MCV=mean corpuscular volume; MCHC=mean corpuscular hemoglobin concentration; RDW=red cell distribution width; TSAT= transferrin saturation; TIBC=total iron binding capacity; sTfR=soluble transferrin receptor; MMA= methylmalonic acid; CRP=C-reactive protein; ESR=erythrocyte sedimentation rate; AI=anemia of inflammation; ALT=alanine transferase; AST=aspartate transferase; ALP=alkaline phosphatase; GGT=gamma glutamyl transpeptidase; INR=international normalized ratio; eGFR=estimated glomerular filtration rate; G6PD=glucose-6-phosphate dehydrogenase; LDH=lactate dehydrogenase; DAT=direct antiglobulin test.

The status/presence of clinical characteristics, complications and medication exposures were determined at each hemoglobin measurement timepoint for the purposed of regression analysis. Children were labelled as having SIBO at the time of hemoglobin measurement if they had symptoms consistent with SIBO on review of clinic notes within 1 month of the date of lab measurements or had a positive duodenal culture or histologic evidence supportive of a diagnosis of SIBO within 3 months of the date of hemoglobin measurement. Vitamin and micronutrient deficiencies measured within 6 months of the hemoglobin measurement were considered present at the time of hemoglobin measurement, as these investigations are typically only done every 6 months. Histologic inflammation, AU and staple line ulcers were considered present if they were identified on endoscopy and histology within 6 months of the hemoglobin measurement timepoint. A 6-month window was considered reasonable given that these histologic and endoscopic findings typically take several months to resolve with treatment (if they do resolve) and are likely present for several months prior to their identification as endoscopy is usually only done for investigation of persistent anemia despite treatment. If the ulcers had been established as being chronic over time after repeat endoscopy and had not responded to treatment, they were considered present at all timepoints following their initial diagnosis. The same was true for liver disease determined by the presence of histologic cirrhosis. Given that not all variables were assessed at the time of the hemoglobin measurement or within the window of time described above for specific variables, additional variables were created for univariable regression analysis if there was “any history” of that specific variable/risk factor, specifically liver disease, histologic inflammation, and GI bleed. This was done in an effort to overcome these

temporal limitations inherent in retrospective research and to assess if these variables they could still be important predictors of anemia.

3.3 Definitions

Outcome Variables:

Anemia: Serum hemoglobin level $\geq$ 2 standard deviations below normal for age and sex (Table 2-1, appendix).

Iron Deficiency Anemia (IDA): Ferritin < 30 μg/L **or** TSAT < 15% in the presence of microcytic anemia (Table 6-A).

*** IDA classification using soluble transferrin receptor (sTfR):** Ferritin < 30 μg/L **and** either sTfR > normal for age **or** sTfR-F index > 1.5 (Table 7-A).

Anemia of Inflammation (AI): Normocytic or microcytic anemia with ferritin > 100 μg /L and TSAT low or normal in the presence of evidence of inflammation (Table 6-A). Evidence of inflammation includes elevated serum CRP, ESR, FCP or evidence of intestinal inflammation on histology. Normal reference values are reported in Table 2-1 (appendix).

***Anemia of Inflammation classification using soluble transferrin receptor (sTfR):** Ferritin > 100μg/L **and** sTfR $\leq$ normal for age **or** sTfR-F index $\leq$ 1.5 in the presence of evidence of inflammation (Table 7-A).

**A secondary classification scheme which removes the need for evidence of inflammation to permit for sub-biochemical subclinical inflammation was utilized as described in section 3.4 and in Table 6-B and Table 7-B.

Mixed IDA and AI: Normocytic or microcytic anemia with ferritin >30 μg /L and < 100 μg /L and TSAT < 20% in the presence of evidence of inflammation (elevated serum CRP, ESR, fecal calprotectin or evidence of intestinal inflammation on histology) (Table 6-A).

***Mixed IDA and AI classification using soluble transferrin receptor (sTfR):** Ferritin > 30 μg /L **and** sTfR > normal for age **or** sTfR-F index > 1.5 (Table 7-A).

**A secondary classification scheme which removes the need for evidence of inflammation to permit for sub-biochemical subclinical inflammation was utilized as described in section 3.4 and in Table 6-B and Table 7-B.

Nutritional anemia: Macrocytic anemia in the presence of folate deficiency (folate < 12.2 nmol/L) or vitamin B12 deficiency (B12 < 155 pmol/L or MMA > 0.4 μmol/L).[148]

Exposure Variables:

Intestinal Failure (IF): Critical reduction of the gut mass or its function below the minimum needed to absorb nutrients and fluids required for adequate growth in children for a minimum of 60 days within a 74 consecutive day interval.[149]

Short Bowel Syndrome (SBS): the consequence of any natural loss or surgical resection of small bowel.[2]

Parenteral Nutrition (PN): intravenous administration of water, macronutrients (glucose, lipids, amino acids), electrolytes, minerals, and micronutrients (trace elements and vitamins).[149]

Iron Deficiency: Serum ferritin < 30 μg /L or TSAT < 15%. In presence of inflammation (systemic or intestinal – described below), serum ferritin < 100 μg /L or serum ferritin >100 μg /L and TSAT < 20%.[38]

Vitamin/Micronutrient Deficiency: Defined by serum micronutrient level below the lower limit of normal specific to that micronutrient. Normal reference values for vitamins A, D and E, copper, ceruloplasmin, zinc, folate, vitamin B12 and MMA are found in Table 2-1 (appendix).

Small Intestinal Bacterial Overgrowth (SIBO): a diagnosis of SIBO can be made based on clinical symptoms, including an increase in the following above baseline that is not otherwise explained and responds to antibiotic therapy: abdominal distention, flatulence, increased stool/ostomy outputs, watery stools, foul-smelling stools, or d-lactic acidosis. A positive hydrogen breath test (increase in hydrogen $\geq$ 20 parts per million [ppm] above baseline within 90 mins of ingestion of 75 grams (g) glucose or 10g lactulose) or positive small bowel aspirate and culture (> 10^3 colony forming units/milliliters [CFU/mL] of bacteria) are also diagnostic of SIBO, if these procedures have been performed by the primary physician.[150] SIBO was also considered to be present if histologic findings consistent with SIBO, specifically villous blunting with or without increased intraepithelial lymphocytosis or increased chronic inflammatory cells in the lamina propria, were present at time of endoscopy in the presence of clinical symptoms of SIBO and if histologic findings could not be explained by another etiology, such as celiac disease, acute enteric infection or non-steroidal anti-inflammatory drug (NSAID) use.[151,152]

Intestinal Inflammation: Fecal calprotectin > 150 ug/g or histologic evidence of active or chronic inflammation on intestinal biopsy, as reported by the pathologist.[153]

Systemic Inflammation: Investigations supportive of the presence of systemic inflammation included elevated CRP or ESR above normal reference range for age (Table 2-1, appendix) which is not otherwise explained by acute injury or infection.

Chronic Kidney Disease: GFR < 60 mL/min/1.73m^2 or persistent proteinuria $\geq$ 3 months. GFR will be estimated using bedside Schwartz equation; GFR (mL/min/1.73 m^2) = (0.41 × Height in cm) / Creatinine in mg/dL.[154] Staging of CKD is per the Kidney Disease: Improving Global Outcomes (KIDGO) organization guidelines (Table 3-1, appendix).[155]

Intestinal Failure Associated Liver Disease (IFALD): Persistent elevation of direct bilirubin > 34 μmol/L (2 mg/dL) for minimum of 2-4 weeks in the context of intestinal failure, or evidence of cirrhosis/fibrosis, inflammation, or portal hypertension (as determined by liver doppler and/or

low platelets +/- low albumin and splenomegaly) or chronic cholestasis on ultrasound, liver elastography or liver biopsy.[89,156]

3.4 Classification of Anemia Types

Determination of nutritional anemia, which we defined as a macrocytic anemia in the presence of folate deficiency or vitamin B12 deficiency in our study, was straightforward. However, classification and differentiation of IDA, AI and mixed IDA/AI is challenging and no one laboratory test is sufficient to define iron deficiency, especially in the presence of inflammation. Anemia types (IDA, AI and mixed IDA/AI) were determined using a combination of MCV, ferritin, TSAT and evidence of inflammation for participants followed in Alberta as previously described (Table 3-A).[24,38] The GIFT team does not routinely use TSAT and instead relies on a combination of sTfR and ferritin to assess iron status and thus, a combination of these investigations was used for classification of anemia types for participants followed by the GIFT team in Toronto, as previously described (Table 4-A).[157] Unfortunately, sTfR testing is not available in Alberta.

When these primary classification schemes were utilized with our study data, more than 75% of the anemic hemoglobin measurements could not be classified because they did not fit the classification schemes or had missing lab parameters. After careful review of the patterns of hemoglobin indices, iron studies, inflammatory markers, concomitant complications (such as liver disease, SIBO or micronutrient deficiencies), presence of iron supplementation or recent PRBC transfusion in each of the unclassified anemia measurements for participants followed by the CHIRP teams in Alberta, it was observed that most of the unclassified anemia measurements would fit into categories of AI or mixed IDA/AI but did not have evidence of inflammation as defined by elevated CRP, ESR, FCP or they had not undergone endoscopic evaluation and thus,

there was no histologic evidence of inflammation to refer to. The majority of children were on oral iron supplementation and thus we contemplated if the oral iron supplementation could have resulted in a normocytic anemia with ferritin >30 and thus, been indicative of IDA. After review of the literature and consultation with a hematologist, it was determined that the response of isolated IDA to oral iron supplementation would result in correction of the hemoglobin prior to normalization of the MCV and ferritin would be the last marker of iron status to normalize. This would indicate that any normocytic anemia with ferritin <100 ug/L is a mixed IDA/AI. The sensitivity of CRP, ESR and FCP in detecting systemic inflammation and for use in the classification of anemia types in the pediatric IF population has not been explored at length. The lack of elevation in CRP or ESR in spite of established intestinal inflammation is seen in other conditions of intestinal inflammation, such as IBD, where some individuals may generate inflammation through pathways not mediated by CRP and have documented intestinal inflammation without an elevated CRP or ESR.[158–161] This phenomenon of normal CRP levels despite evidence of intestinal inflammation has also been described in a recent study in children with IF with chronic intestinal inflammation resembling IBD; all 23 children with histologically confirmed IBD-like intestinal inflammation had normal CRP levels.[93] While missing FCP data was the main hindrance to its use in anemia classification, there are conflicting reports on the utility of FCP in detecting intestinal inflammation in children with IF and/or SIBO.[74,93,162,163]

Therefore, after careful analysis of the pattern of labs and consideration of the documented literature, we created a secondary classification scheme for the types of anemia which removes the need for a marker of inflammation for AI and mixed IDA/AI and refer to these types as AI or mixed IDA/AI with sub-biochemical subclinical inflammation (Table 3-B). The range for ferritin was

also expanded for mixed IDA/AI based on our results, which are described in section 4.2. A similar pattern of classification was encountered when using the primary classification scheme for the participants from Toronto and given that sTfR is considered a sensitive marker to discriminate between isolated IDA and AI and mixed IDA/AI, we also created a secondary classification scheme to allow for sub-biochemical subclinical inflammation without the need for an elevated CRP, ESR or FCP (Table 4-B).

Table 3-A: Primary classification scheme for iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed iron deficiency anemia/anemia of inflammation based on routine iron studies

Anemia type	MCV (fL)*	Ferritin (μg /L)	Transferrin saturation (TSAT) (%)	Evidence of inflammation**
Iron deficiency anemia (IDA)	Low	< 30	< 15%	No
Anemia of inflammation (AI)	Low or normal	> 100	Low or normal	Yes
Mixed IDA/AI	Low or normal	30-100	< 20%	Yes

Adapted from Capellini et al. (2017). * MCV normal values for age listed in Table 2-1 in appendix **elevated CRP, ESR or FCP. MCV = mean corpuscular volume; CRP = c-reactive protein; ESR = erythrocyte sedimentation rate; FCP = fecal calprotectin

Table 3-B: Secondary classification scheme for iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed iron deficiency anemia/anemia of inflammation based on routine iron studies allowing for sub-biochemical subclinical inflammation

Anemia	MCV (fL)*	Ferritin (μg /L)	Transferrin saturation (TSAT) (%)	Evidence of inflammation**
Iron deficiency anemia (IDA)	Low	< 30	< 15%	No
Anemia of inflammation (AI)	Low or normal	> 100	Low or normal	Yes
Anemia of inflammation – subclinical inflammation	Low or normal	>100	Low or normal	**No**
Mixed IDA/AI	Normal	0-100	< 20%	Yes
Mixed IDA/AI – subclinical inflammation	Normal	0-100	< 20%	**No**
			OR	
	Low	30-100	<20%	**No**

* MCV normal values for age listed in Table 2-1 in appendix. **elevated CRP, ESR or FCP. MCV = mean corpuscular volume; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; FCP = fecal calprotectin

Table 4-A: Primary classification scheme for iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed iron deficiency anemia/anemia of inflammation based on soluble transferrin receptor (sTfR) and ferritin studies

Anemia	Ferritin (μg /L)	Soluble transferrin receptor (sTfR) (mg/L)*	Soluble transferrin receptor index (sTfR-F index)	Evidence of Inflammation**
Iron deficiency anemia (IDA)	< 30	> normal for age	> 1.5	No
Anemia of inflammation (AI)	> 100	≤ normal for age	≤ 1.5	Yes
Mixed IDA/AI	30-100	> normal for age	> 1.5	Yes

Adapted from Vázquez-López et al. (2015) and Vázquez-López et al. (2016). *Normal values for age for sTfR listed in Table 2-1 in appendix. **Elevated ESR, CRP or FCP. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; FCP = fecal calprotectin

Table 4-B: Secondary classification scheme for iron deficiency anemia (IDA), anemia of inflammation (AI) and mixed iron deficiency anemia/anemia of inflammation based on soluble transferrin receptor (sTfR) and ferritin studies allowing for sub-biochemical subclinical inflammation

Anemia	Ferritin (μg /L)	Soluble transferrin receptor (sTfR) (mg/L)*	Soluble transferrin receptor index (sTfR-F index)	Evidence of Inflammation**
Iron deficiency anemia (IDA)	< 30	> normal for age	> 1.5	No
Anemia of inflammation (AI)	> 100	≤ normal for age	≤ 1.5	**Not required**
Mixed IDA/AI	0-100	> normal for age	> 1.5	**Not required**

Adapted from Vázquez-López *et al* (2015) and Vázquez-López et al. (2016). *Normal values for age for sTfR listed in Table 2-1 in appendix. **Elevated ESR, CRP or fecal calprotectin.

3.5 Prevalence of Anemia

The period prevalence of anemia was determined by the number of children who had anemia had anytime during the study period divided by the total number of children in the study. The prevalence of each type of anemia was similarly calculated by the number of children who had the specific type of anemia at any time during the study period divided by the total number of children in the study. Some children had more than 1 type of anemia and thus were included in the numerator for more than 1 type of anemia. For this reason, statistical comparisons between anemia types were not performed as it would result in counting some children more than once and would not yield a valid statistical result. A child with any anemia and 2 or more hemoglobin measurement timepoints during the study period was determined to have chronic anemia if they had anemia on 2 or more of their hemoglobin measurement timepoints. Thus, the prevalence of chronic anemia was calculated by the number of children with anemia on 2 or more of their hemoglobin measurement timepoints divided by the total number of children with anemia who had at least 2 hemoglobin measurement timepoints.

3.6 Statistical Analysis

Descriptive statistics were calculated as frequencies for categorical variables and medians with interquartile ranges (IQRs) for continuous variables. Proportions of clinical and demographic variables, as well as prevalence of anemia, chronic anemia and anemia types between groups stratified by anemia status, intestinal rehabilitation center, SBS status or GI bleed status were compared using the Chi square test of independence or Fisher's exact test for categorical variables where appropriate. Fisher's exact test was used in instances where expected

frequencies in cells were <5, as Chi square test is not reliable in those conditions.[164] The Wilcoxon Rank Sum test was used for comparisons of continuous variables, such as age at enrolment and duration of observation. A summary of the variable descriptions and statistical tests used with each exposure and outcome variable is detailed in Table 4-1 (appendix). Statistical analysis assessing differences between IDA, AI and mixed IDA/AI at the participant level were not performed as many children had more than 1 type of anemia during the study period. As a result, these children would be represented in more than 1 group and would be counted twice or even three times, which would not result in meaningful and valid statistical results when trying to assess differences between the groups.

Patient characteristics and outcomes were stratified by anemia status, SBS status, GI bleed status and IRP location (Alberta vs Toronto) to attempt to identify meaningful differences and potential predictors of anemia, as outlined in the exploratory objectives (section 2.2).

We selected a mixed effects logistic regression model to assess for potential predictors of anemia given that the data included repeated measures/panel data. For the regression analysis, each anemia episode was considered a unique event and potential factors were assessed relative to the temporal association related to the unique anemia event. Thus, the regression analysis evaluated event-level data as opposed to patient level data. The outcome was anemia compared to no anemia. This model was also used for each anemia type (IDA, AI, mixed IDA/AI) compared to no anemia. Univariable analysis was performed on all exposure variables/potential associated variables of anemia and each of the types of anemia; the strength of the association and level of uncertainty was expressed as odds ratios (OR) with 95% confidence intervals (CI). A p-value < 0.5

was considered statistically significant. All statistical analysis was performed using STATA® version 16.1 (STATACorp LLC).

4 Chapter 4: Results

4.1 Descriptive Statistics – Demographic and Clinical Characteristics

4.1.1 Participant Characteristics

A total of 90 children (49 [54%] male) with IF were enrolled in the study, including 54 children followed at Toronto SickKids Hospital and 36 children followed by the two IRPs in Alberta; Alberta Children's Hospital (23 children) and Stollery Children's Hospital (13 children). The median age of participants at enrolment was 4.9 (IQR 3.0-9.7) years and the median duration of observation for all participants was 3.9 (IQR 2.2-8.0) years. The median lifetime duration of PN was 4.4 (IQR 2.5-8.5) years; 24 (27%) children had been weaned off PN by enrolment. The baseline demographic and disease specific characteristics are summarized in Table 5. Gastroschisis was the leading cause of IF among participants (28%), followed by necrotizing enterocolitis (14%), intestinal volvulus (12%) and intestinal atresia (10%) (Figure 3). Hirschsprung's disease and other disorders of intestinal motility were the underlying etiology of IF in 8% and 6% of participants respectively, while congenital diarrhea disorders and mucosal enteropathies affected 6% and 4% of participants respectively.

Table 5: Demographic and clinical characteristics of participants by anemia status and anemia type

Participant characteristics	Total cohort (n=90)	Anemia (n=75)	No anemia (n=15)	*p*-value	Iron deficiency anemia (IDA) (n=15)	Anemia of inflammation (AI) (n=40)	Mixed IDA/AI (n=32)
Male, n (%)	49 (54%)	41 (55%)	8 (53%)	0.93	11 (73%)	20 (50%)	18 (56%)
Age (years) at enrolment, median (IQR)	4.9 (3.0-9.7)	5.3 (3.2-10)	3.9 (1.8-8.1)	0.24	5.4 (4.3-10.0)	6.1 (4.0-10.1)	6.1 (3.6-10.5)
Hemoglobin measurement timepoints (number), median (IQR)	4 (2-8)	5 (3-9)	2 (1-4)	**0.003**	5 (4-9)	5 (3-9)	5 (4-9)
Duration of observation (years), median (IQR)	3.9 (2.2-8.0)	4.3 (2.3-8.4)	2.4 (1.1-5.3)	**0.02**	4.8 (3.4-8.0)	4.5 (3.0-8.7)	5.1 (3.1-8.9)
Lifetime PN duration (years), median (IQR)	4.4 (2.5-8.5)	4.8 (2.8-8.8)	2.9 (0.6-6.4)	0.07	5.6 (4.4-8.6)	4.9 (3.5-9.4)	5.0 (2.7-8.8))
Primary etiology of IF				0.36			
Gastroschisis	25 (28%)	21 (28%)	4 (27%)		5 (33%)	10 (25%)	11 (34%)
Necrotizing enterocolitis	13 (14%)	1 (15%)	2 (13%)		3 (20%)	6 (15%)	4 (13%)
Volvulus	11 (12%)	8 (11%)	3 (20%)		0 (0%)	5 (13%)	2 (6%)
Intestinal atresia	9 (10%)	6 (8%)	3 (20%)		2 (13%)	4 (10%)	1 (3%)
Hirschsprung's disease	7 (8%)	7 (9%)	0 (0%)		0 (0%)	5 (13%)	5 (16%)
Congenital diarrhea disorder	6 (7%)	6 (8%)	0 (0%)		1 (7%)	3 (8%)	3 (9%)
Dysmotility	5 (6%)	5 (7%)	0 (0%)		1 (7%)	1 (3%)	2 (6%)
Mucosal enteropathy*	4 (4%)	4 (5%)	0 (0%)		1 (7%)	2 (5%)	2 (6%)
Meconium ileus	3 (3%)	3 (4%)	0 (0%)		0 (0%)	1 (3%)	1 (3%)
Spontaneous perforation	1 (1%)	1 (1%)	0 (0%)		0 (0%)	0 (0%)	0 (0%)
Other	6 (7%)	3 (4%)	3 (20%)		2 (13%)	3 (8%)	1 (3%)
Surgical Characteristics							
Short bowel syndrome (SBS), n (%)	69 (77%)	56 (75%)	13 (87%)	0.32	12 (80%)	30 (75%)	23 (72%)
SBS type[a]				0.62			
SBS type I (end jejunostomy)	1 (1%)	1 (2%)	0 (0%)		0 (0%)	1 (4%)	0 (0%)
SBS type II (jejuno-colic anastomosis)	42 (63%)	35 (65%)	7 (54%		11 (92%)	19 (68%)	14 (61%)
SBS type III (jejuno-ileocolic anastomosis)	25 (37%)	18 (33%)	6 (46%)		1 (8%)	8 (29%)	9 (39%)
Percent predicted small bowel length, median (IQR)[b]	24 (14-42)	21 (13-42)	27 (29-38)	0.10	21 (11-37)	19 (11-42)	24 (19-38)
Ileocecal valve present, n (%)[a]	28 (41%)	20 (36%)	8 (62%)	0.09	1 (8%)	8 (27%)	16 (44%)
History of STEP, n (%)[a]	19 (28%)	14 (25%)	5 (38%)	0.33	3 (25%)	6 (20%)	7 (30%)

Table 5: Demographic and clinical characteristics of participants by anemia status and anemia type… continued

Participant characteristics	Total cohort (n=90)	Anemia (n=75)	No anemia (n=15)	*p*-value	Iron deficiency anemia (IDA) (n=15)	Anemia of inflammation (AI) (n=40)	Mixed IDA/AI (n=32)
IF-associated complications							
Liver disease, n (%)	37 (41%)	31 (41%)	6 (40%)	0.92	8 (53%)	18 (45%)	9(28%)
Chronic kidney disease, n (%)	0 (0%)	0 (0%)	0 (0%)	-	0 (0%)	0 (0%)	0 (0%)
GI Bleed, n (%)	22 (24%)	20 (27%)	2 (13%)	0.34	6 (40%)	13 (33%)	12 (38%)
SIBO, n (%)	36 (40%)	31 (41%)	5 (33%)	0.56	9 (60%)	15 (38%)	19 (59%)
Elevated fecal calprotectin, n (%)[c]	3 (15%)	3 (18%)	3 (15%)	1.00	0 (0%)	3 (50%)	3 (20%)
Endoscopic characteristics							
Endoscopic assessment, n (%)	39 (43%)	36 (48%)	3 (20%)	0.05	10 (67%)	18 (45%)	26 (81%)
Histologic inflammation, n (%)[d]	24 (62%)	22 (61%)	2 (67%)	1.00	8 (80%)	9 (50%)	14 (54%)
Histologic evidence of SIBO, n (%)[d]	16 (41%)	15 (42%)	1 (33%)	1.00	5 (50%)	9 (50%)	10 (38%)
Anastomotic ulcer, n (%)[d]	8 (21%)	7 (19%)	1 (33%)	0.51	3 (30%)	4 (22%)	5 (19%)
Staple line ulcer, n (%)[e]	4 (36%)	3 (33%)	1 (50%)	1.00	1 (33%)	2 (67%)	2 (33%)
Medications and supplementation							
Oral iron supplementation	49 (54%)	43 (57%)	6 (40%)	0.22	12 (80%)	18 (45%)	26 (81%)
Iron-supplemented PN	48 (53%)	39 (52%)	9 (60%)	0.57	7 (47%)	25 (63%)	5 (16%)
Intravenous iron treatment	10 (11%)	39 (52%)	0 (0%)	0.20	4 (22%)	7 (18%)	7 (22%)
Packed red blood cell transfusion	20 (22%)	20 (27%)	0 (0%)	**0.02**	5 (33%)	10 (25%)	10 (31%)
SIBO antibiotic therapy/prophylaxis	36 (40%)	31 (41%)	5 (33%)	0.56	8 (53%)	14 (35%)	20 (63%)
Proton pump inhibitor (PPI) use	77 (86%)	65 (87%)	12 (80%)	0.50	13 (87%)	38 (95%)	23 (72%)

*Mucosal enteropathy includes microvillus inclusion disease and tufting enteropathy

[a] n= 67 for total cohort, n=54 for anemia, n=13 for no anemia, n=12 for IDA, n=28 for AI, n=23 for Mixed IDA/AI . [b] n= 83 for total cohort, n=69 for anemia and n=14 for no anemia, n= 12 for IDA, n=37 for AI, n=29 for Mixed IDA/AI. [c] n= 20 for total cohort, n=17 for anemia, n=20 for no anemia, n=3 for IDA, n=6 for AI, n=4 for Mixed IDA/AI. [d]n=39 for total cohort, n=36 for anemia, n=3 for no anemia, n=10 for IDA, n=18 for AI, n=26 for Mixed IDA/AI. [e] n= 11 for total cohort (includes those with SBS, history of STEP and history of endoscopy), n= 9 for anemia, n= 2 for no anemia, n=3 for IDA, n=3 for AI, n=6 for mixed IDA/AI.

IDA = iron deficiency anemia; AI = anemia of inflammation; PN = parenteral nutrition; IF = intestinal failure; SBS = short bowel syndrome; STEP= serial transverse enteroplasty; GI = gastrointestinal; SIBO = small intestine bacterial overgrowth; PPI=proton pump inhibitor.

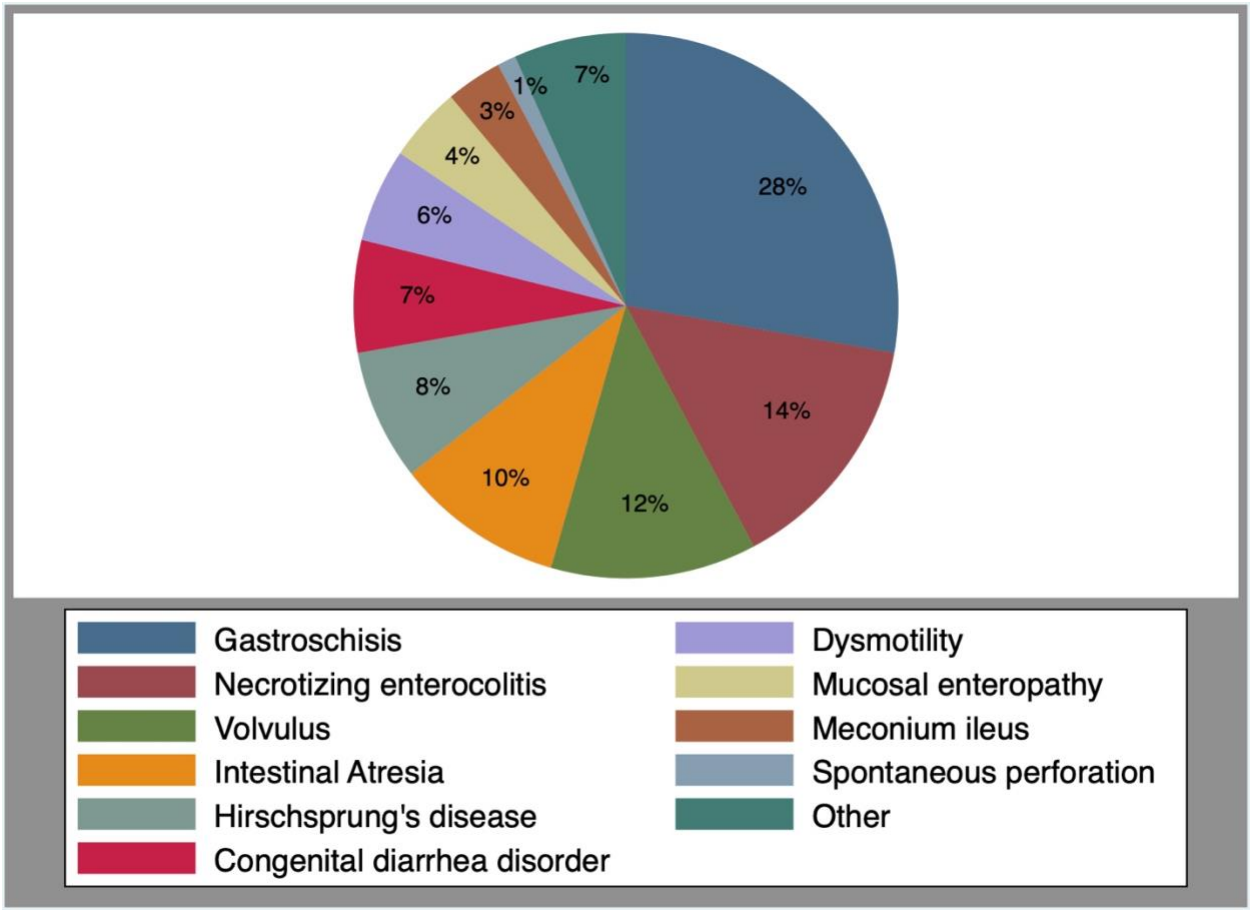

Figure 3. Primary etiology of intestinal failure of study participants.

4.1.2

4.1.2 Short Bowel Syndrome and Surgical and Anatomic Characteristics

A total of 69 (77%) children had congenital or surgical resection of the small bowel resulting in a requirement for PN and thus met diagnostic criteria for SBS. Sixty-five children with SBS had small bowel length measurements at time of surgery with a median percent predicted small bowel length of 24 (IQR 14-42) percent; 33 children (51%) had a percent predicted small bowel length of less than 25%. The majority of children with SBS had SBS type II anatomy (jejuno-colic anastomosis) (42 [63%]), while 24 (36%) had SBS type III (jejuno-ileocolic anastomosis) and only 1 child had SBS type I anatomy (enterostomy). The ileocecal valve was present in 28 (41%) of children with SBS. A total of 19 (28%) children with SBS underwent STEP procedures; 9 children had a repeat STEP procedure following the first STEP and 2 of those 9 children underwent a third STEP procedure.

4.1.3 Intestinal Failure-Associated Complications and Endoscopic Findings

IFALD and SIBO were the most common IF related complications; IFALD was present in 37 (41%) children and SIBO was present in 36 (40%) children (Table 5). None of the participants had chronic kidney disease. Twenty-two (24%) children had one or more episodes of overt gastrointestinal bleeding presenting as hematemesis, hematochezia or melena. A total of 26 fecal calprotectin tests were performed in 20 children during the study period; 3 (15%) of these children had elevated FCP levels on one or more occasions. The primary etiology of IF in these children with elevated FCP levels varied; one child had a history of necrotizing enterocolitis and was later diagnosed with IBD at age 7 years; one child had a history of gastroschisis and STEP procedure, and the third child with elevated FCP had IF secondary to dysmotility.

A total of 39 (43%) children underwent endoscopy (esophagogastroduodenoscopy [EGD] and/or colonoscopy) during the study period, and 1 child also underwent capsule endoscopy to help determine the underlying cause of their anemia. Histologic inflammation was common and was present in 24 (62%) children who underwent endoscopy. Sixteen of thirty-nine (41%) children who underwent endoscopy had endoscopic and histologic findings supporting their clinical diagnosis of SIBO, specifically duodenal/small bowel mucosal erosions and ulcers, villous blunting, and intraepithelial lymphocytosis; 7 of these children also had cultures of small bowel aspirates obtained during 8 endoscopic procedures which further supported the diagnosis of SIBO. A total of 27 children with a history of SBS underwent endoscopy and 7 (26%) were found to have anastomotic ulcers. Of the 11 children who underwent endoscopy and had a history of STEP, 4 (36%) had staple line ulcers identified on endoscopy.

4.1.4 Medications and Supplements

Most children received iron supplementation, either prophylactic or therapeutic, at some time during the study observation period, including oral iron supplementation in 49 (54%) children, IV iron therapy in 10 (11%) children and PN supplemented with iron in 48 (53%) children (Table 5). Twenty (22%) children received PRBC transfusions for anemia during the study. Antibiotics for prophylactic or therapeutic management of SIBO were used in 36 (40%) of children, and 77 (86%) used PPIs at some point during the study period.

4.2 Anemia

4.2.1 Frequency of Anemia and Anemia Types

A total of 448 hemoglobin measurements were obtained from the 90 participants, of which 227 (51%) met age-based criteria of anemia. In Toronto, 103 of 225 hemoglobin measurements (46%) met age-based criteria of anemia compared to 124 of 223 in Alberta (56%) (p=0.04). MCV was normal for age in 79% of the measures of anemia (normocytic anemia), low in 16% (microcytic anemia) and high in 6% (macrocytic anemia).

Using the primary classification scheme for anemia types based on MCV, ferritin, TSAT and biochemical evidence of inflammation for participants from Alberta (Table 3-A), only 27 hemoglobin measurements were classified as either IDA (13 [11%]), AI (10 [8%]) or mixed IDA/AI (4 [3%]); 75 measurements (60%) did not fit the classification scheme ("other" anemia) and 22 measurements (18%) were unclassified due to missing lab parameters, such as ferritin, MCV, TSAT, CRP, ESR or FCP (Table 8-A). Using the secondary anemia classification scheme (Table 3-B), which allows for sub-biochemical subclinical inflammation and expands the range of ferritin for mixed IDA/AI, an additional 63 hemoglobin measurements were able to be classified into an anemia type (Table 6-A). Mixed IDA/AI was the most frequent type of anemia comprising 53 (43%) (including 40 [32%] mixed IDA/AI with sub-biochemical subclinical inflammation) of anemia measurements, followed by AI with 24 (19%) (including 13 [11%] with sub-biochemical subclinical inflammation). IDA was the least frequent type comprising 13 (11%) anemia measurements.

The primary classification scheme for anemia types for participants from Toronto based on sTfR, sTfR-F index and biochemical evidence of inflammation (elevated CRP, ESR or FCP) (Table 4-A) resulted in the classification of 40 of 103 anemic hemoglobin measurements as either IDA

(13 [13%]), AI (23 [22%]) or mixed IDA/AI (4 [4%]); 24 [23%] were classified as "other" anemia because of inability to fit the classification scheme while an additional 40 measurements (39%) could not be classified due to missing lab parameters, such as ferritin, CRP, ESR or FCP. The secondary classification scheme (Table 4-B), which allowed for sub-biochemical subclinical inflammation by removing the need for additional markers of inflammation (elevated CRP, ESR or FCP) for AI and mixed IDA/AI resulted in the classification of an additional 23 hemoglobin measurements (Table 6-B). The most common type of anemia was AI (44 [43%]), followed by IDA (13 [13%]) and finally mixed IDA/AI (5 [4%]); 11 (11%) were classified as other anemia and 30 (29%) remained unclassified. **Note, the remainder of the manuscript will report data obtained using the secondary classification schemes for classification of anemia types, unless otherwise specified.**

Combining the data from both intestinal rehabilitation centers (Alberta and Toronto), of the 227 hemoglobin measurements that were anemic for age, the classification of the anemia types was as follows: 26 (11%) were classified as IDA, 68 (30%) AI and 58 (26%) mixed IDA/AI. Twenty-three (10%) hemoglobin measurements were classified as other anemia and 52 (23%) remained unclassified due to missing lab values.

Twenty-four children were weaned off PN by the end of the observation period. While only 34 hemoglobin measurements were collected while children were off PN compared to 414 measurements collected while on PN, the frequency of anemia was higher in measurements taken while on PN vs off PN; 52% of hemoglobin measurements obtained in children on PN were anemic compared to 32% of hemoglobin measurements obtain while off PN (p=0.03).

Table 6-A: Frequency of anemia types for participants followed in Alberta by classification criteria scheme

Anemia type (n=124 anemic hemoglobin measurements)	**Primary classification scheme[a]**	**Secondary classification scheme[b]**
Iron deficiency anemia (IDA)	13 (11%)	13 (11%)
Anemia of inflammation (AI)	10 (8%)	10 (8%)
AI with sub-detectable inflammation	n/a	14 (11%)
Mixed IDA/AI	4 (3%)	13 (11%)
Mixed IDA/AI with sub-detectable inflammation	n/a	40 (32%)
Other anemia*	75 (60%)	12 (10%)
Unclassified due to missing lab parameters	22 (18%)	22 (18%)

[a] as per Table 3-A. [b] as per Table 3-B.

*Other anemia refers to instances of anemia where the pattern of labs do not fit the anemia classification scheme. Unclassified due to missing lab parameters refers to instances of anemia where a key lab parameter needed for the classification of the type of anemia is missing, *i.e.,* ferritin, MCV, TSAT, CRP, ESR.

Table 6-B: Frequency of anemia types for participants followed in Toronto by classification criteria scheme

Anemia type (n=103 anemic hemoglobin measurements)	**Primary classification scheme[a]**	**Secondary classification scheme[b]**
Iron deficiency anemia (IDA)	13 (13%)	13 (13%)
Anemia of inflammation (AI)	23 (22%)	44 (43%)
Mixed IDA/AI	4 (4%)	5 (5%)
Other anemia*	24 (23%)	11 (11%)
Unclassified due to missing lab parameters	40 (39%)	30 (29%)

[a] as per Table 4-A. [b] as per Table 4-B.

*Other anemia refers to instances of anemia where the pattern of labs do not fit the anemia classification scheme. Unclassified due to missing lab parameters refers to instances of anemia where a key lab parameter needed for the classification of the type of anemia is missing, *i.e.,* ferritin, sTfR.

4.2.2 Prevalence of Anemia

The 10-year period prevalence of anemia was 83%, with 75 children having anemia on at least 1 annual hemoglobin measurement. Of the 72 participants with anemia and two or more hemoglobin measurement timepoints, 55 (76%) had chronic anemia, defined as anemia on 2 or more annual hemoglobin measurements. There was no difference in participant characteristics in children with anemia versus without anemia, with the exception that children with anemia had more hemoglobin measurement timepoints (5 vs 2; p=0.003) and a longer duration of observation (4.3 vs 2.4 years; p=0.02) (Table 5).

4.2.3 Prevalence of Anemia Types

Figure 4 depicts both the period prevalence of anemia and types of anemia, as well as the prevalence of chronic anemia for the total cohort of participants and by IRPs (Toronto vs Alberta). Of the 3 major classes of anemia, IDA had the lowest prevalence for the entire cohort at 17%, followed by mixed IDA/AI at 36% and finally AI was the most common type at 44%. The prevalence of “other” anemia, those anemias which did not fit the classification scheme, was 19%. None of the participants had nutritional anemia. A total of 19 (26%) participants had more than 1 type of anemia during the study (Figure 5). Participant characteristics by anemia type are shown in Table 5. Statistical analysis to evaluate for differences between anemia types was not performed given that many children had >1 type of anemia during the observation period and thus were represented in more than one anemia type group.

Figure 6 depicts the anemia status for each patient at each CBC measurement timepoint during the study period and visually demonstrates the chronic and recurrent nature of anemia, as well as evolution between anemia types in some children. Of the 227 hemoglobin measurements which were anemic for age, 102 (45%) of these instances of anemia occurred while children were on oral iron supplementation (depicted by white x" in Figure 6). Meanwhile, 89/227 (39%) anemic hemoglobin measurements occurred in children receiving iron-supplemented PN at the time of their anemia (depicted by shaded boxes in Figure 6).

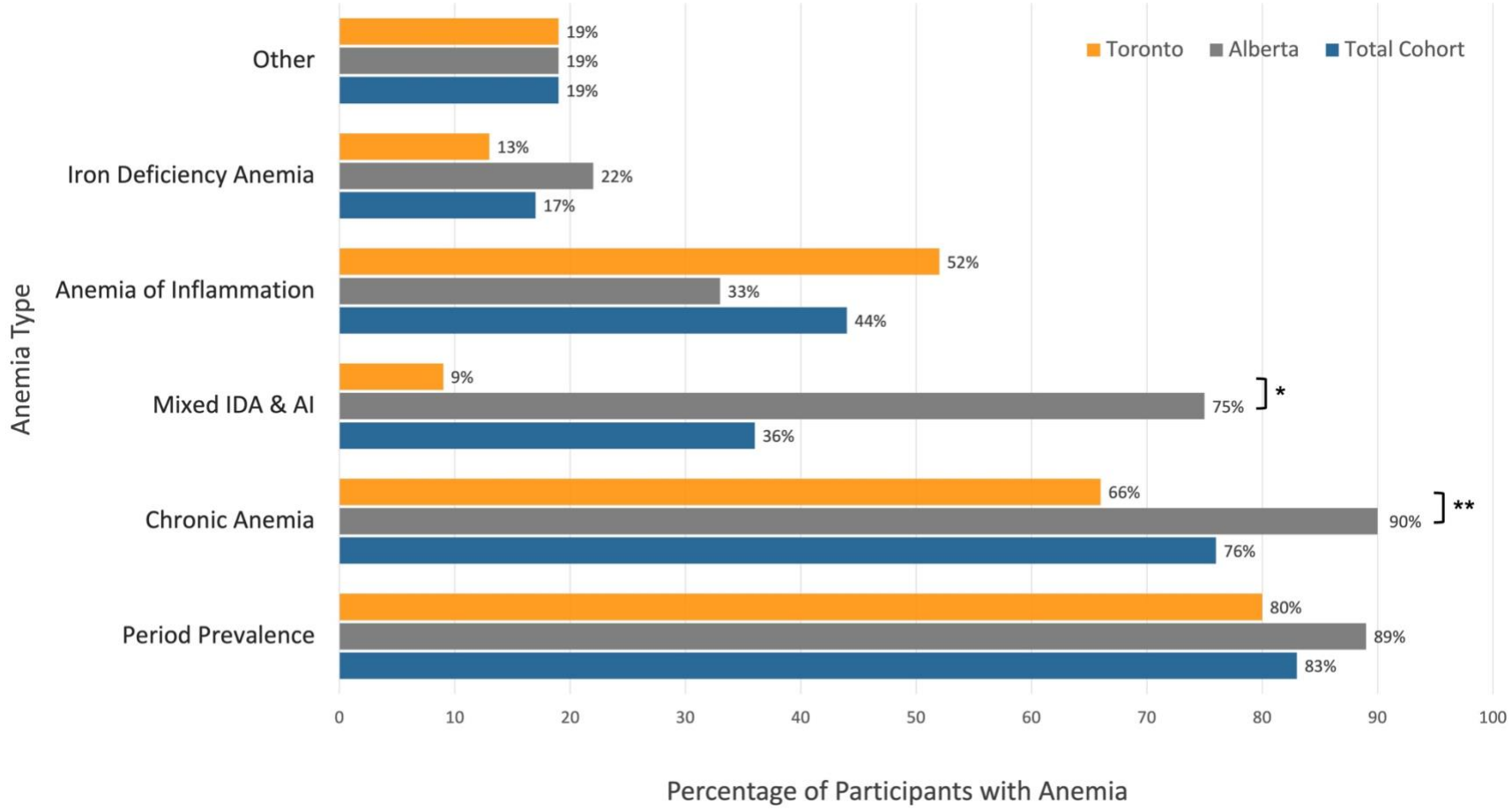

Figure 4. Period prevalence of anemia and its types for the total study cohort and by intestinal rehabilitation program location.

Other anemia refers to cases of anemia which did not fit the classification scheme for IDA, AI or Mixed IDA/AI. Chronic anemia is defined as having anemia on two or more hemoglobin measurements in the study period; prevalence of chronic anemia was determined by using only those patients with anemia and two or more hemoglobin measurement timepoints in the study. There were no episodes of nutritional anemia. * *p*-value < 0.001; ** *p*-value=0.02.

IDA=iron deficiency anemia; AI=anemia of inflammation.

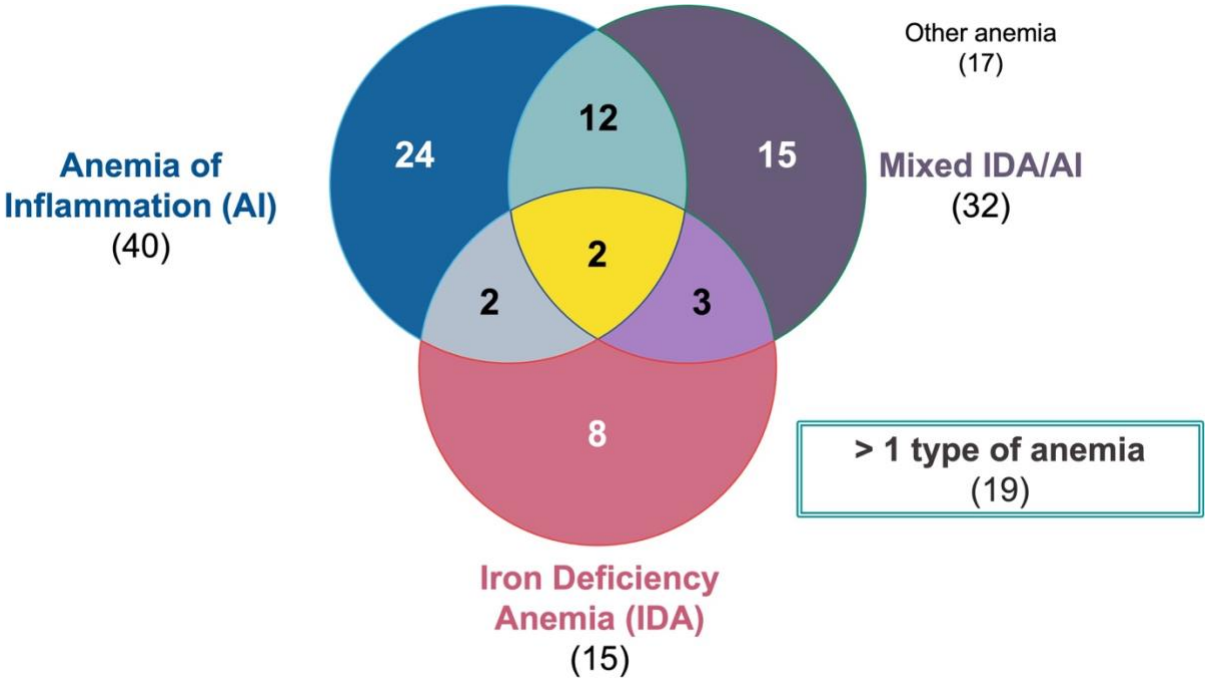

Figure 5. Venn diagram depicting the number of participants which each type of anemia at any time during the study period.

Total n=90 participants. Other anemia refers to anemia which did not fit the classification scheme for IDA, AI or Mixed IDA/AI.

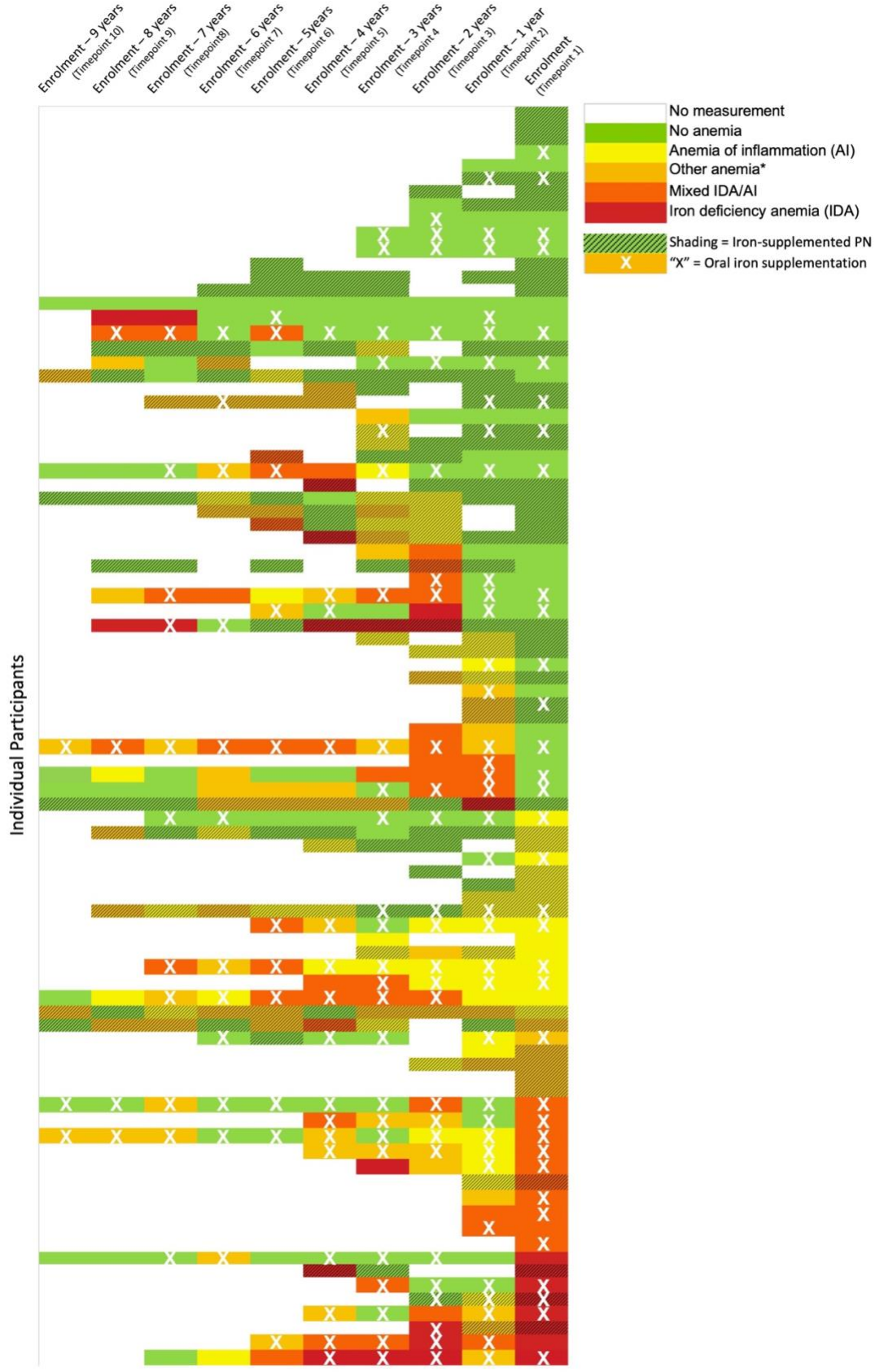
Enrolment – 9 years (Timepoint 10)
Enrolment – 8 years (Timepoint 9)
Enrolment – 7 years (Timepoint8)
Enrolment – 6 years (Timepoint 7)
Enrolment – 5years (Timepoint 6)
Enrolment – 4 years (Timepoint 5)
Enrolment – 3 years (Timepoint 4
Enrolment – 2 years (Timepoint 3)
Enrolment – 1 year (Timepoint 2)
Enrolment (Timepoint 1)
No measurement
No anemia
Anemia of inflammation (AI)
Other anemia*
Mixed IDA/AI
Iron deficiency anemia (IDA)
Shading = Iron-supplemented PN
"X" = Oral iron supplementation
Individual Participants

Figure 6. Heat map depicting presence of anemia and anemia type at each hemoglobin measurement timepoint for each participant over the observation period.

Each horizontal row along the y-axis represents an individual participant. The x-axis represents each annual hemoglobin measurement timepoint. The most recent measurement at time of enrolment in the study (timepoint 1) is aligned along the right side of the x-axis and each prior annual measurement is shown in increasing years from enrolment when moving right to left along the x-axis. Boxes are colour-coded for anemia status at each hemoglobin measurement timepoint, with white boxes indicating no available hemoglobin measurement. Due to the varying age of participants at enrolment, some participants have only one annual hemoglobin measurement and thus have only one colored box, while some children have up to 10 years of data and have 10 boxes. White boxes interspersed between colored boxes indicate no available annual hemoglobin measurement for that year for that participant. Shaded boxes represent hemoglobin measurements taken while participants were on parenteral nutrition supplemented with iron. “White X” represents participants who were on oral iron supplementation at the time of hemoglobin measurement.

4.3 Associated Vitamin and Micronutrient Deficiencies

4.3.1 Prevalence of Associated Vitamin and Micronutrient Deficiencies

Figure 7 depicts the prevalence of vitamin and micronutrient deficiencies in children with IF. Iron deficiency was the most common deficiency with 67% of children having at least 1 deficient measurement during the study period. None of the children had vitamin D deficiency (serum vitamin D < 25 nmol/L), but Vitamin D insufficiency (serum vitamin D 25-50 nmol/L) was common and was found in 52% of children. Zinc (10%), ceruloplasmin (8%), vitamin B12 (8%) and copper (7%) deficiencies were less common. Folate deficiency was only observed in 2 children (2%) and neither child had a history of any anemia during the study. Vitamin B12 deficiency was present on 10 measurements among 7 children. Only 2/10 measures of vitamin B12 deficiency occurred with concomitant anemia, including a mixed IDA/AI and an unclassified normocytic anemia type (missing TSAT) and thus, there were no episodes of isolated nutritional anemia. Apart from folate deficiency, there was no statistically significant difference in prevalence of micronutrient deficiencies between children with and without anemia, including no difference in the prevalence of iron deficiency.

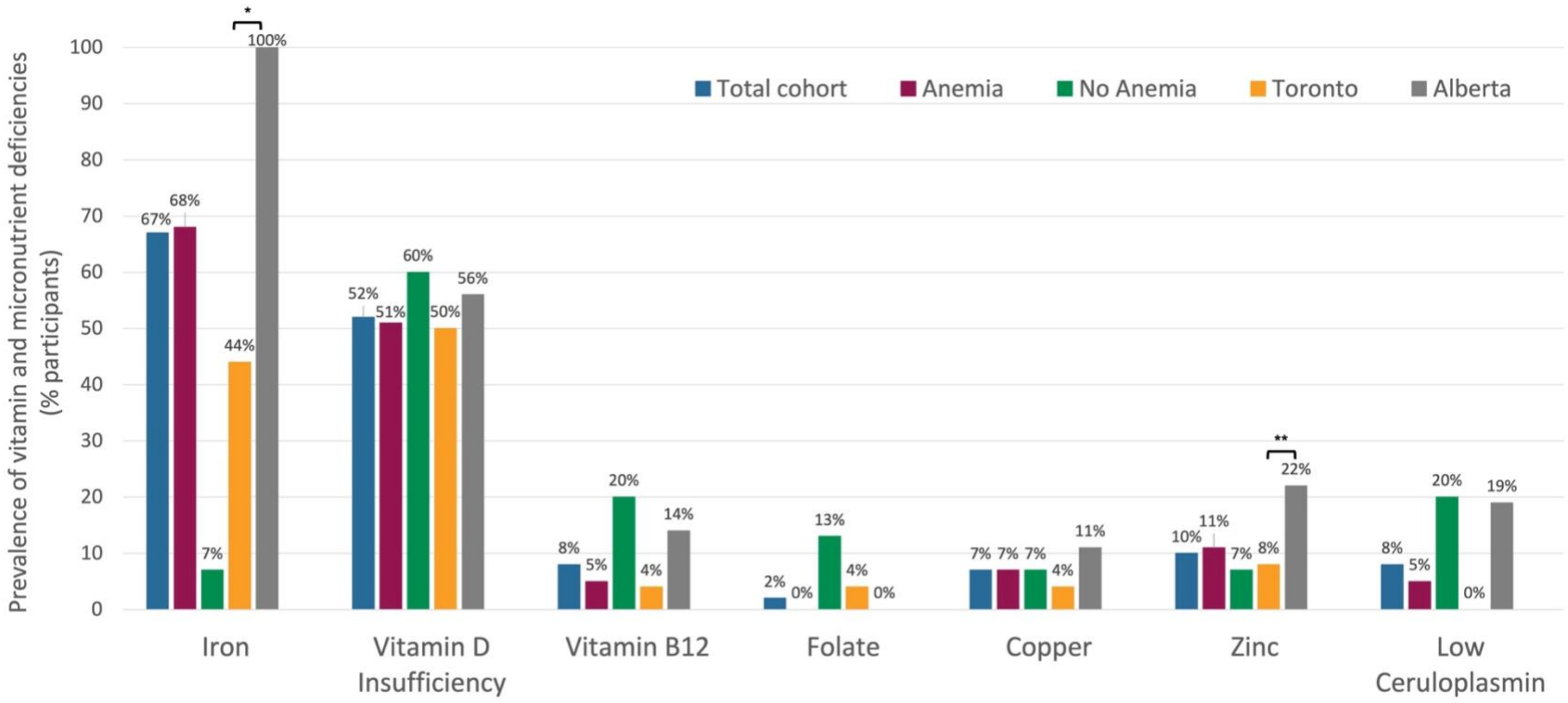

Figure 7. Period prevalence of vitamin and micronutrient deficiencies in children with intestinal failure during the study period.

* $p<0.001$; ** $p=0.002$.

Vitamin D insufficiency is displayed; there were no participants with vitamin D deficiency.

4.4 Sub-analysis

4.4.1 Between Intestinal Rehabilitation Program Center Analysis

4.4.1.1 Participant Characteristics

Having identified the use of iron-supplemented PN by the IRP in Toronto as a unique practice difference in comparison to the IRPs in Alberta, a post-hoc sub-analysis was performed to assess how this practice difference could impact anemia, anemia types and IF-associated complications. There was no difference in the primary etiology of IF between children followed in Toronto versus Alberta (Table 7). Participants ranged in age from 9 months to 17.9 years at study enrolment. When comparing age at enrolment, as well as duration of observation between Toronto and Alberta, the distributions between the groups were similar, but the *p*-values approached statistical significance; the median age at enrolment was 4.7 (IQR 2.6-9.6) years in Toronto versus 5.8 (IQR 3.8-10.5) years in Alberta (p=0.05), while median duration of observation was 3.5 (IQR 1.8-6.9) years in Toronto compared to 4.8 (IQR 3.1-8.8) years in Alberta (p=0.05). There was a positive association between age at enrolment and number of hemoglobin measurement timepoints (p=0.01) (Figure 8) and thus, the number of hemoglobin measurement timepoints was lower in Toronto (median 3 [IQR 2-6]) versus Alberta (median 6 [IQR 2-10]; p=0.001). The distribution of hemoglobin measurement timepoints in Toronto compared to Alberta is depicted in Figure 9.

Table 7: Participant characteristics and prevalence of anemia by intestinal rehabilitation program center

Participant characteristics	Total cohort (n=90)	Toronto (n=54)	Alberta (n=36)	*p*-value
Male, n (%)	49 (54%)	29 (54%)	20 (56%)	0.86
Age (years) at enrolment, median (IQR)	4.9 (3.0-9.7)	4.7 (2.6-9.6)	5.8 (3.8-10.5)	**0.05**
Hemoglobin measurement timepoints (number), median (IQR)	4 (2-8)	3 (2-6)	6 (2-10)	**0.001**
Duration of observation (years), median (IQR)	3.9 (2.2-8.0)	3.5 (1.8-6.9)	4.8 (3.1-8.8)	**0.05**
Lifetime PN duration (years), median (IQR)	4.4 (2.5-8.5)	3.8 (2.0-7.9)	4.9 (1.7-8.8)	0.25
Primary etiology of IF				0.16
Gastroschisis	25 (28%)	11 (20%)	14 (39%)	
Necrotizing enterocolitis	13 (14%)	9 (17%)	4 (11%)	
Volvulus	11 (12%)	8 (15%)	3 (8%)	
Intestinal atresia	9 (10%)	8 (15%)	1 (3%)	
Hirschsprung's disease	7 (8%)	2 (4%)	5 (14%)	
Congenital diarrhea disorder	6 (7%)	5 (9%)	1 (3%)	
Dysmotility	5 (6%)	2 (4%)	3 (8%)	
Mucosal enteropathy*	4 (4%)	3 (6%)	1 (3%)	
Meconium ileus	3 (3%)	2 (4%)	1 (3%)	
Spontaneous perforation	1 (1%)	0 (0%)	1 (3%)	
Other	6 (7%)	4 (7%)	2 (6%)	
Anemia				
Period prevalence of anemia, n (%)	75 (83%)	43 (80%)	32 (89%)	0.25
Chronic anemia, n (%)	55 (76%)[a]	27 (66%)[b]	28 (90%)[c]	**0.02**
Anemia types				
Iron deficiency anemia (IDA)	15 (17%)	7 (13%)	8 (22%)	0.25
Anemia of inflammation (AI)	40 (44%)	28 (52%)	12 (33%)	0.08
Mixed IDA and AI	32 (36%)	5 (9%)	27 (75%)	**<0.001**
Other[#]	17 (19%)	10 (19%)	7 (19%)	0.91
More than 1 type of anemia	19 (26%) [a]	5 (12%) [b]	14 (45%) [c]	**0.02**
Surgical characteristics				
Short bowel syndrome (SBS)	69 (77%)	40 (70%)	29 (81%)	0.28
SBS type[d]				0.89
SBS type I (end jejunostomy)	1 (1%)	1 (3%)	0 (0%)	
SBS type II (jejuno-colic anastomosis)	42 (62%)	23 (59%)	19 (66%)	
SBS type III (jejuno-ileocolic anastomosis)	25 (37%)	15 (38%)	10 (34%)	
Percent predicted small bowel length, median (IQR)[e]	24 (14-42)	25 (11-55)	24 (19-37)	0.95
Ileocecal valve present, n (%)[d]	28 (41%)	17 (43%)	11 (38%)	0.70
History of STEP, n (%)[d]	19 (28%)	10 (25%)	9 (31%)	0.58

Table 7: Participant characteristics and prevalence of anemia by intestinal rehabilitation program center…continued

Participant characteristics	Total cohort (n=90)	Toronto (n=54)	Alberta (n=36)	*p*-value
IF-associated complications				
Liver disease, n (%)	37 (41%)	28 (52%)	9 (25%)	**0.01**
Chronic kidney disease, n (%)	0 (0%)	0 (0%)	0 (0%)	-
GI bleed, n (%)	22 (24%)	8 (15%)	14 (39%)	**0.01**
SIBO, n (%)	36 (40%)	15 (28%)	21 (58%)	**0.004**
Elevated fecal calprotectin, n (%)	3/20 (15%)	-	3/20 (15%)	-
Endoscopic characteristics				
Endoscopic assessment, n (%)	39 (43%)	12 (22%)	27 (75%)	**<0.001**
Histologic inflammation, n (%)	24 (62%)[f]	6 (50%)[g]	18 (67%)[h]	0.32
Histologic evidence of SIBO, n (%)	18 (46%)[f]	6 (50%)[g]	12 (44%)[h]	0.75
Anastomotic ulcers, n (%)	8 (21%)[f]	1 (8%)[g]	7 (26%)[h]	0.39
Staple line ulcers, n (%)	4 (36%)[i]	1 (33%)[j]	3 (38%)[k]	1.00
Medications/supplementation				
Oral iron supplementation	49 (54%)	16 (30%)	33 (92%)	**<0.001**
Iron-supplemented PN	48 (53%)	48 (89%)	0 (0%)	**<0.001**
Intravenous iron treatment	10 (11%)	2 (4%)	8 (22%)	**0.01**
Packed red blood cell transfusion	20 (22%)	11 (20%)	9 (25%)	0.61
SIBO antibiotic therapy/prophylaxis	36 (40%)	15 (28%)	21 (58%)	**0.004**
Proton pump inhibitor (PPI) use	77 (86%)	52 (96%)	25 (69%)	**<0.001**

[#]Other includes those with anemia which did not fit the classification parameters for IDA, AI or Mixed anemia, or, had missing data required to classify the anemia, such as ferritin or sTfR. *Mucosal enteropathy includes microvillus inclusion disease and tufting enteropathy.

[a] n=72, [b] n=41, [c] n=31, # of those with anemia and > 1 hemoglobin measurement. [d] n= 69 for total cohort, n=40 for Toronto, n=29 for Alberta. [e] n= 83 for total cohort, n= 52 for Toronto, n=31 for Alberta. [f] n= 39, [g] n= 12, [h] n= 27. [i]n=11, [j] n= 3, [k] n= 8.

PN = parenteral nutrition; IF = intestinal failure; SBS = short bowel syndrome; STEP= serial transverse enteroplasty; GI = gastrointestinal; SIBO = small intestine bacterial overgrowth; PPI = proton pump inhibitor.

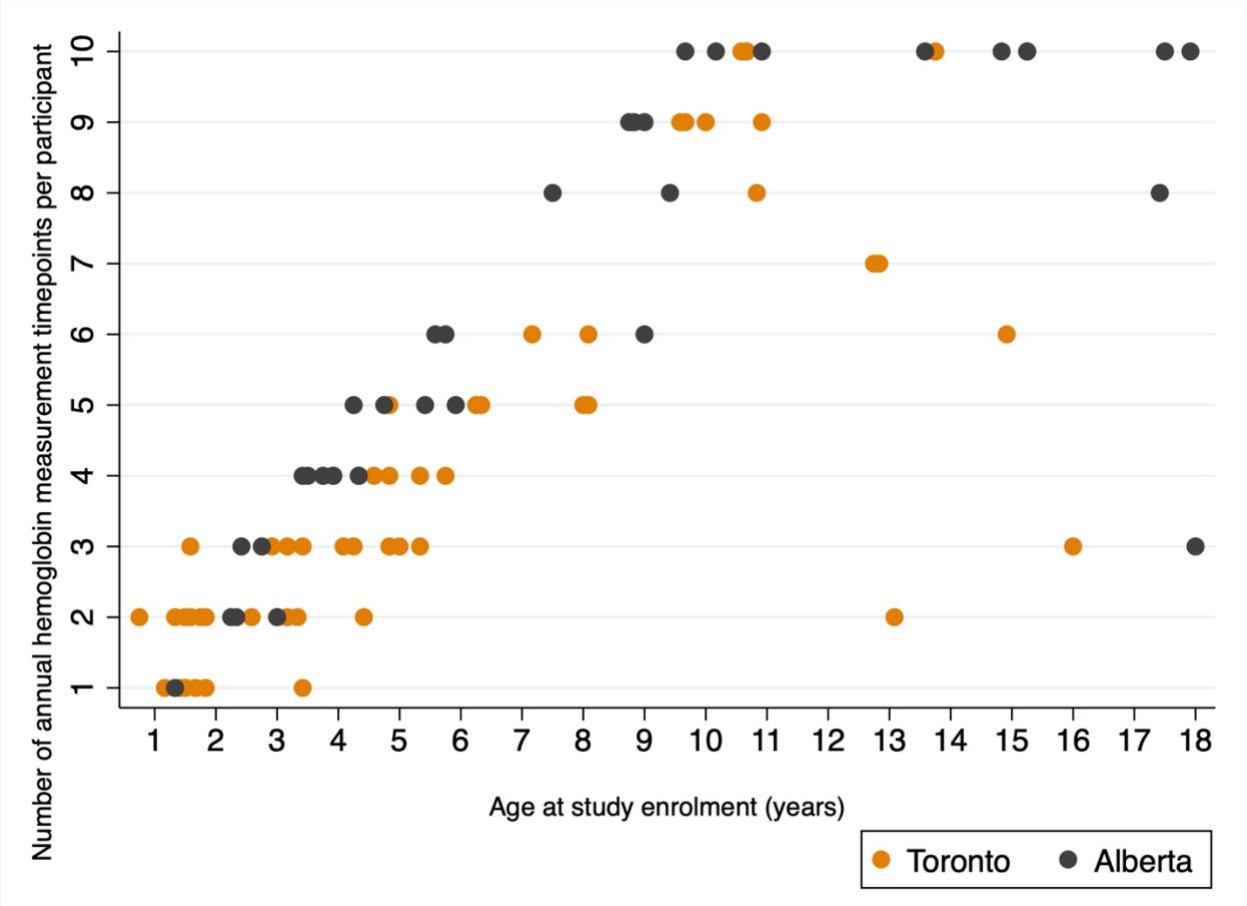

Figure 8. Scatterplot illustrating positive correlation between participant age at enrolment and number of annual hemoglobin measurement timepoints.

Each data point represents an individual participant. The location of the intestinal rehabilitation program (IRP) the participant is followed by (Toronto or Alberta) is depicted by colour of data point. Alberta includes two IRPs, located in Edmonton and Calgary.

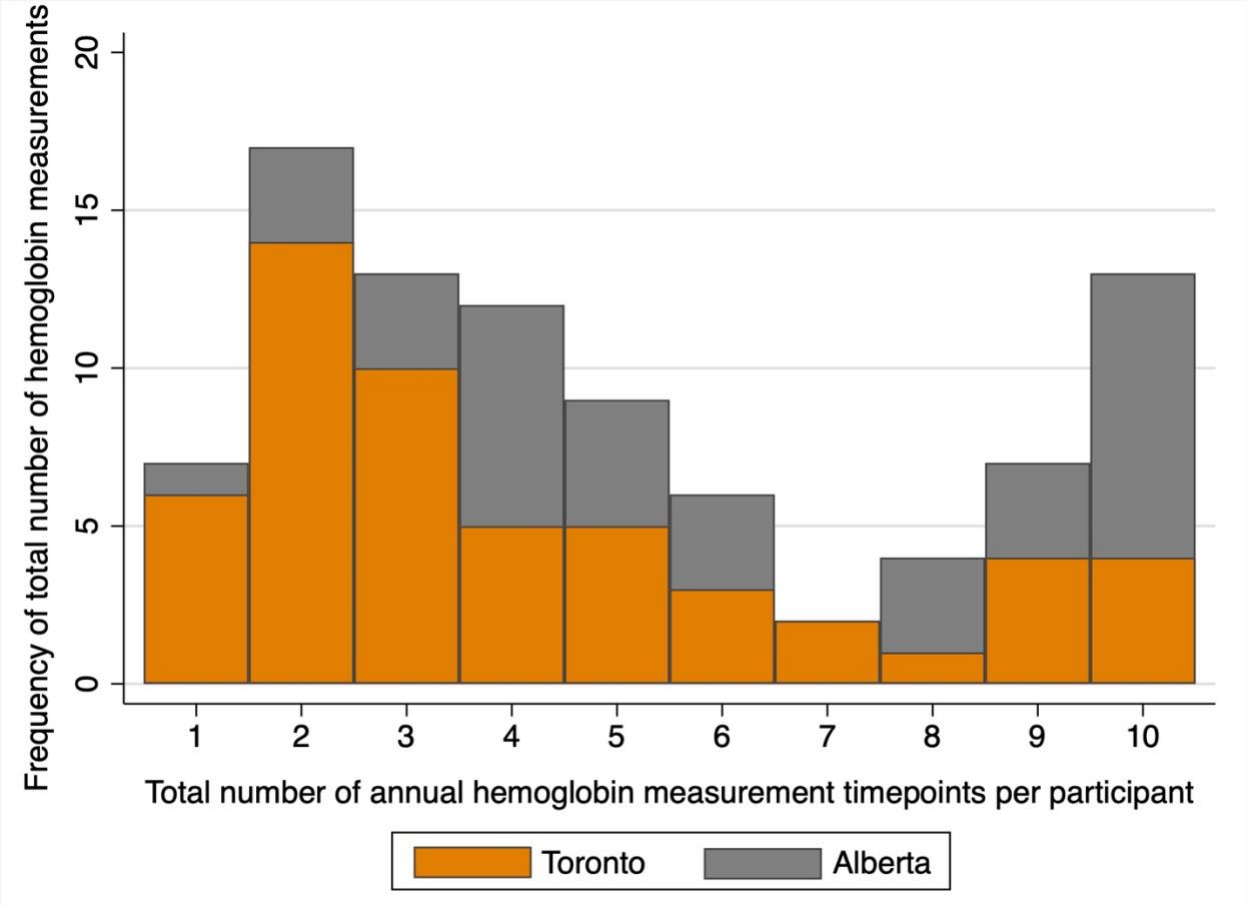

Figure 9. Histogram demonstrating the frequency of distribution of the number of annual hemoglobin measurement timepoints by intestinal rehabilitation program (IRP) location. Alberta includes two IRPs located of Edmonton and Calgary.

4.4.1.2 IF-Associated Complications and Endoscopic Findings

Between site comparisons of the prevalence of IF-associated complications revealed a higher prevalence of liver disease in Toronto compared to Alberta (52% vs 25%; p=0.01), while the frequency of GI bleeds was lower in Toronto compared to Alberta (8 [15%] vs 14 [39%]; p=0.01), as was the frequency of SIBO (15 [28%] vs 21 [58%]; p=0.004) (Table 7).

With respect to measures of intestinal inflammation, all 20 children who had FCP tests performed were followed by the intestinal rehabilitation teams in Alberta, and thus, between site comparisons could not be performed. While a lower number of children underwent endoscopy in Toronto compared to Alberta (12 [22%] vs 27 [75%]; p<0.001), there was no statistically significant difference in the prevalence of histologic intestinal inflammation in children followed in Toronto vs Alberta (50% vs 67%, p=0.32). There were no differences in the frequency of histologic evidence supporting a diagnosis of SIBO between children in Toronto vs Alberta (6 [50%] vs 12 [44%]; p=0.75), nor was there a difference with respect to the frequency of anastomotic ulcers (1 [8%] vs 7 [26%]; p=0.39) or staple line ulcers (3 [33%] vs 3 [38%]; p=1.00) (Table 7).

4.4.1.3 Medications

There were statistically significant differences between Toronto and Alberta with regards to the forms of iron supplementation used. The use of iron-supplemented PN is a routine practice in Toronto but is not used at all in Alberta and thus, most children followed in Toronto received iron-supplemented PN (48 [89%]) while no children in Alberta had access to it (p<0.001) (Table 7). In contrast, oral and IV iron supplementation/treatment was less common in Toronto; 16 (30%)

of children received oral iron supplementation compared to compared to 33 (92%) in Alberta (p<0.001) and 2 (4%) children in Toronto received IV iron infusions compared to 8 (22%) in Alberta (p<0.001). Use of antibiotics for SIBO treatment or prophylaxis was lower in Toronto (15 [28%]) compared to Alberta (21 [58%]; p=0.004), while PPI use was higher in Toronto vs Alberta (52 [96%] vs 25 [69%]; p<0.001).

4.4.1.4 Prevalence of Anemia and Anemia Types

There was no difference in the period prevalence of anemia between IRPs in Toronto compared to Alberta (period prevalence 80% vs 89%; p=0.25). However, the prevalence of chronic anemia was lower in Toronto compared to Alberta (66% vs 90%; p=0.02) (Table 7/Figure 4). The prevalence of IDA showed a trend towards lower prevalence in Toronto compared to Alberta, but this did not reach statistical significance (13% vs 22%; p=0.25) while the opposite trend occurred with AI with a non-significantly higher prevalence in Toronto compared to Alberta (52% vs 33%; p=0.08). There was a statistically significant lower prevalence of mixed IDA/AI in Toronto compared to Alberta (9% vs 75%; p <0.001) (Table 7/Figure 4). The prevalence of "other" anemia was stable at 19% across the entire cohort and between IRP locations.

4.4.1.5 Micronutrient Deficiencies and Iron Status

The period-prevalence of iron deficiency was significantly less frequent in children with IF managed in Toronto compared to Alberta (44% vs 100%; p<0.001), as was zinc deficiency (8% vs 22%; p=0.002) (Figure 7). There were no between site differences with respect to the

prevalence of vitamin D insufficiency or deficiencies of vitamin B12, folate, or copper. There were no measures of serum ceruloplasmin obtained from children followed in Toronto.

Evaluation of the accompanying iron indices collected at time of hemoglobin measurements revealed that 48% (166) of the 346 hemoglobin measurements collected during the study period that had accompanying measures of iron status were consistent with iron deficiency. In comparing rates of iron deficiency by IRP location, Toronto had lower rates of iron deficiency compared to Alberta; 43 of 186 (23%) of hemoglobin measurement timepoints with accompanying iron indices were iron deficient in Toronto compared to 123 of 160 (77%) in Alberta ($p<0.001$). Iron deficiency with anemia occurred in 29% (100/346) of hemoglobin/iron indices measurements and iron deficiency without anemia occurred in 19% (66/346) of measurements ($p=0.15$). When examining only those hemoglobin measurements with iron deficiency, 60% (100/166) occurred with anemia compared to 40% (66/166) of measurements or iron deficiency without anemia ($p=0.01$). Ferritin levels differed between centers with a median of 257 ug/l, (IQR 60-434) in Toronto compared to median 68 ug/L, (IQR 25-300) in Alberta ($p<0.001$) (Figure 10). Toronto had a greater number of ferritin measurements > 200 ug/L in comparison to Alberta (61% [126/208] vs 8% [15/200]) ($p=0.001$). Accompanying TSAT measurements were not available in Toronto to assess if the elevated ferritin levels were in keeping with inflammation or if they were consistent with iron overload. However, when comparing ferritin levels in children based on iron supplementation in PN, median ferritin levels were higher in the children on iron-supplemented PN in instances of both anemia and no anemia (Figure 11). For those without anemia, median ferritin level was 310 (IQR 134-481) ug/L in children receiving iron-supplemented PN compared to median 46 (IQR 25-72) ug/L in children receiving PN without iron ($p<0.001$). Similarly in cases

of anemia, median ferritin level was 255 (IQR 32-410) ug/L in children receiving iron-supplemented PN compared to 29 (IQR 14-115) ug/L in children receiving PN without iron (p<0.001).

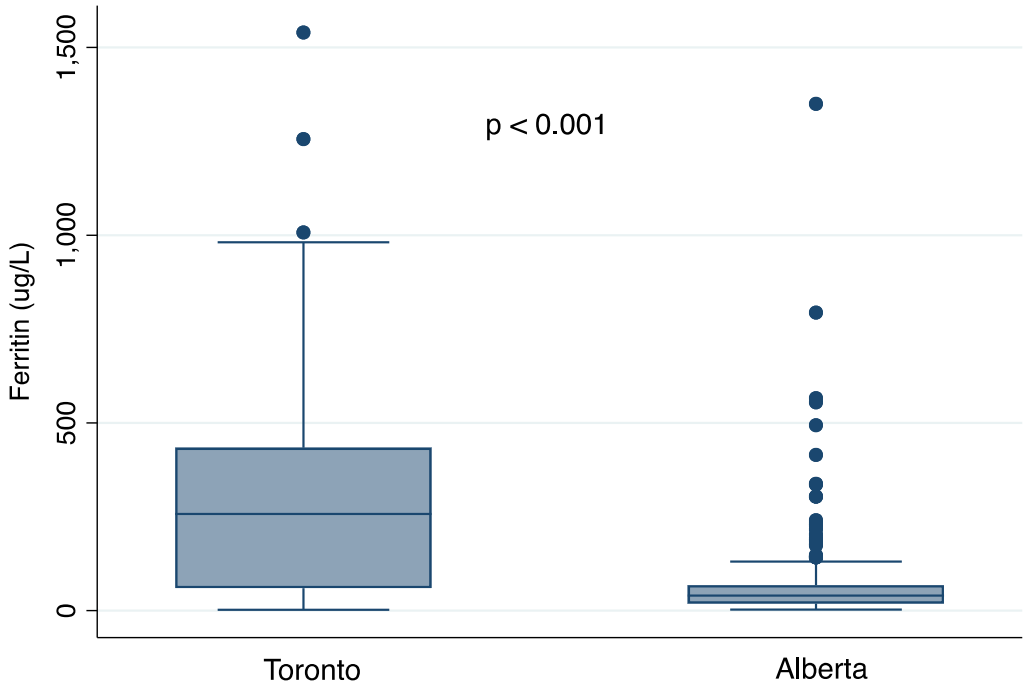

Figure 10. Serum ferritin levels in children with IF followed in Toronto vs Alberta.

Boxplot comparing distribution of serum ferritin levels between children with IF followed in Toronto compared to children with IF followed in Alberta. All ferritin lab values from all timepoints are included.

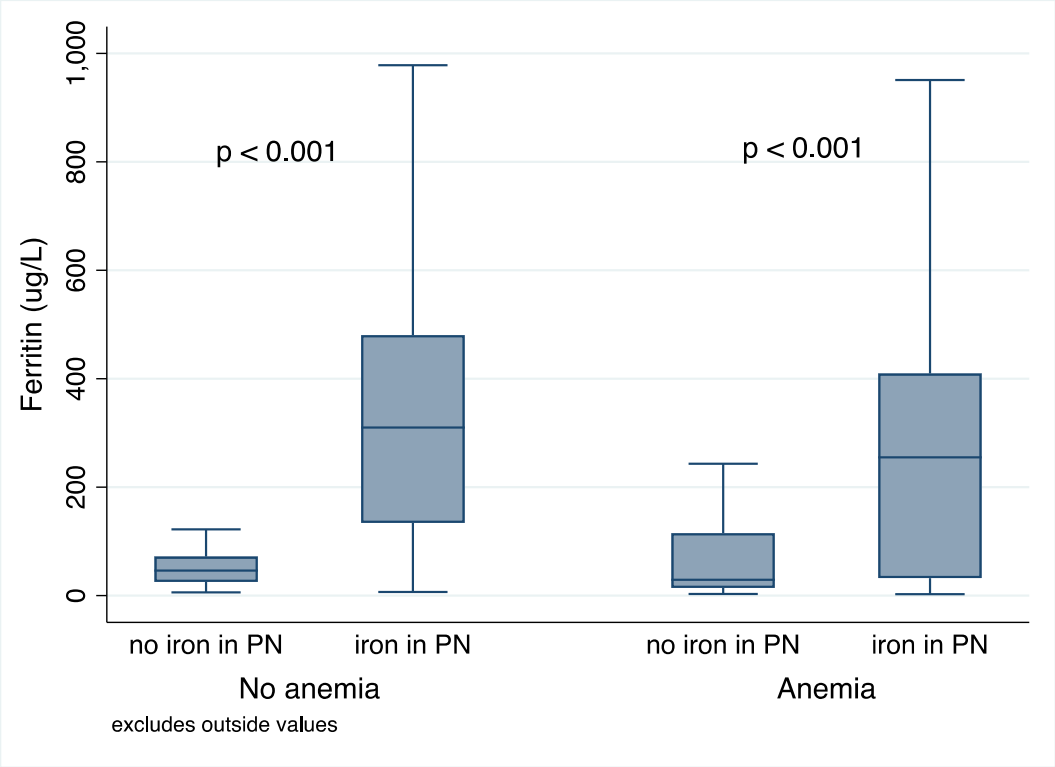

Figure 11. Serum ferritin levels in children with IF based on presence/absence of anemia and iron supplementation in parenteral nutrition (PN).

Boxplot comparing distribution of serum ferritin levels between children with IF who receive iron-supplemented PN to children who receive PN without iron, stratified by anemia status. All ferritin lab values from all timepoints are included

4.4.2 Stratification by Anemia Status, IF-Associated Complications and SBS Status

Some notable differences in IF-associated complications were identified when patients were stratified by history of GI bleed status. The prevalence of SIBO was higher in those with a history of GI bleeds (64%) compared to those with no history of GI bleeds (32%; *p*=0.009) (Table 8). The prevalence of histologic inflammation was higher in those with a history of GI bleeds (61% vs 19%; *p*=0.01), as was the prevalence of anastomotic ulcers (39% vs 5%; *p*=0.02). Interestingly, all 3 children with elevated FCP levels experienced GI bleeds. Unfortunately, only 1 of these 3 children had FCP measurements performed at the time of their GI bleeds, but FCP levels were high at the time of presentation with GI bleeding. There was no difference in the overall period prevalence of anemia in children with a history of GI bleeds compared to those without any GI bleeds (91% vs 81%; *p*=0.34). However, those with a history of GI bleed had a higher prevalence of chronic anemia (95% vs 69%; *p*=0.02), mixed IDA/AI (55% vs 29%; *p*=0.03) and more than 1 type of anemia (48% vs 17%; *p*=0.01) (Table 8). Interestingly, there was no difference in the frequency of histologic inflammation in children with anemia (22/36, [61%]) compared to those without anemia (2/3 [67%]; *p*=1.00). However, endoscopy was performed in 36 (48%) children with anemia compared to only 3 (20%) in children without anemia and this difference approached statistical significance (*p*=0.05), and thus, the small number of scopes (3) in the group without anemia may limit interpretation (Table 5).

There was no difference in the prevalence of anemia, chronic anemia or anemia types when stratified by SBS status, nor were there any differences in baseline demographic and clinical characteristics (Table 9).

Table 8: Participant characteristics and prevalence of anemia by history of gastrointestinal (GI) bleed

Participant characteristics	Total cohort (n=90)	GI bleed (n=22)	No GI bleed (n=68)	*p*-value
Male, n (%)	49 (54%)	12 (55%)	37 (54%)	0.99
Age (years) at enrolment, median (IQR)	4.9 (3.0-9.7)	4.3 (2.4-10.7)	4.7 (3.0-8.1)	**0.03**
Duration of observation (years), median (IQR)	3.9 (2.2-8.0)	8.1 (3.4-9.3)	3.4 (2.1-6.0)	**0.002**
Lifetime PN duration (years), median (IQR)	4.4 (2.5-8.5)	8.8 (4.3-9.9)	3.7 (2.3-6.4)	**0.006**
Primary etiology of IF				0.84
Gastroschisis	25 (28%)	9 (41%)	16 (24%)	
Necrotizing enterocolitis	13 (14%)	3 (14%)	10 (15%)	
Volvulus	11 (12%)	2 (9%)	9 (13%)	
Intestinal atresia	9 (10%)	2 (9%)	7 (10%)	
Hirschsprung's disease	7 (8%)	1 (5%)	6 (9%)	
Congenital diarrhea disorder	6 (7%)	0 (0%)	6 (9%)	
Dysmotility	5 (6%)	2 (9%)	3 (4%)	
Mucosal enteropathy*	4 (4%)	1 (5%)	3 (4%)	
Meconium ileus	3 (3%)	0 (0%)	3 (4%)	
Spontaneous perforation	1 (1%)	0 (0%)	1 (3%)	
Other	6 (7%)	2 (9%)	4 (6%)	
Anemia				
Period prevalence of anemia, n (%)	75 (83%)	20 (91%)	55 (81%)	0.34
Chronic anemia, n (%)	55 (76%)[a]	19 (95%)[b]	36 (69%)[c]	**0.02**
Hemoglobin measurement timepoints (number), median (IQR)	4 (2-8)	9 (4-10)	4 (2-6)	**< 0.001**
Anemia types				
Iron deficiency anemia (IDA)	15 (17%)	6 (27%)	9 (13%)	0.13
Anemia of inflammation (AI)	40 (44%)	13 (59%)	27 (40%)	0.11
Mixed IDA and AI	32 (36%)	12 (55%)	20 (29%)	**0.02**
Other[#]	17 (19%)	4 (18%)	13 (19%)	0.92
More than 1 type of anemia	19 (26%) [a]	10 (50%) [b]	9 (17%) [c]	**0.008**
Surgical characteristics				
Short bowel syndrome (SBS)	69 (77%)	17 (77%)	52 (76%)	0.94
SBS type[d]				0.33
SBS type I (end jejunostomy)	1 (1%)	0 (0%)	1 (2%)	
SBS type II (jejuno-colic anastomosis)	42 (62%)	13 (76%)	29 (57%)	
SBS type III (jejuno-ileocolic anastomosis)	25 (37%)	4 (24%)	21 (41%)	
Percent predicted small bowel length, median (IQR)[e]	24 (14-42)	24 (11-69)	34 (20-91)	0.30
Ileocecal valve present, n (%)[d]	28 (41%)	4 (24%)	24 (46%)	0.10
History of STEP, n (%)[d]	19 (28%)	7 (41%)	12 (23%)	0.15

Table 8: Participant characteristics and prevalence of anemia by history of gastrointestinal (GI) bleed…continued

Participant characteristics	Total cohort (n=90)	GI bleed (n=22)	No GI bleed (n=68)	*p*-value
IF-associated complications				
Liver disease, n (%)	37 (41%)	10 (45%)	27 (40%)	0.63
Chronic kidney disease, n (%)	0 (0%)	0 (0%)	0 (0%)	-
SIBO, n (%)	36 (40%)	14 (64%)	22 (32%)	**0.009**
Elevated fecal calprotectin	3/20 (15%)	3/11 (27%)	0 (0%)	0.22
Endoscopic characteristics				
Endoscopic assessment, n (%)	39 (43%)	18 (82%)	21 (31%)	**<0.001**
Histologic inflammation, n (%)	24 (62%)[e]	13 (72%)[f]	8 (38%)[g]	**0.03**
Histologic evidence of SIBO, n (%)	18 (46%)[e]	11 (61%)[f]	4 (19%)[g]	**0.01**
Anastomotic ulcers, n (%)	8 (21%)[e]	7 (39%)[f]	1 (5%)[g]	**0.02**
Staple line ulcers, n (%)	4 (36%)[h]	4 (52%)[i]	0 (0%)[j]	0.19
Medications/supplementation				
Oral iron supplementation	49 (54%)	14 (64%)	35 (68%)	0.32
Iron-supplemented PN	48 (53%)	8 (36%)	40 (51%)	0.07
Intravenous iron treatment	10 (11%)	5 (23%)	5 (7%)	**0.05**
Packed red blood cell transfusion	20 (22%)	13 (59%)	7 (10%)	**< 0.001**
SIBO antibiotic therapy/prophylaxis	36 (40%)	13 (59%)	23 (34%)	**0.04**
Proton pump inhibitor (PPI) use	77 (86%)	19 (86%)	58 (85%)	0.91

[#]Other includes those with anemia which did not fit the classification parameters for IDA, AI or Mixed anemia, or, had missing data required to classify the anemia, such as ferritin or sTfR. *Mucosal enteropathy includes microvillus inclusion disease and tufting enteropathy. Histologic evidence of SIBO includes mucosal ulcers or erosions, villous blunting and intraepithelial lymphocytosis in the presence of clinical symptoms of SIBO.

[a] n=72, [b] n=20, [c] n=52, # of those with anemia and > 1 hemoglobin measurement. [d] n= 68 for total cohort, n=17 for GI bleed, n=51 for no GI bleed. [e] n= 39, [f] n= 18, [g] n= 21. [h] n=11, [i] n= 7, [j] n= 4.

PN = parenteral nutrition; IF = intestinal failure; SBS = short bowel syndrome; STEP= serial transverse enteroplasty; SIBO = small intestine bacterial overgrowth; PPI = proton pump inhibitor.

Table 9: Prevalence of anemia and patient characteristics stratified by short bowel syndrome (SBS) status

Participant characteristics	Total cohort (n=90)	SBS (n=69)	No SBS (n=21)	*p*-value
Male, n (%)	49 (54%)	41 (60%)	8 (38%)	0.09
Age (years) at enrolment, median (IQR)	4 (3.0-9.7)	4.8 (3.2-10)	5.4 (3.0-9.0)	0.79
Duration of observation (years), median (IQR)	3.9 (2.2-8.0)	3.8 (2.3-8.2)	4.3 (1.9-6.4)	0.48
Lifetime PN duration (years), median (IQR)	4.4 (2.5-8.5)	4.4 (2.5-9.1)	4.7 (2.6-6.3)	0.58
Anemia				
Period prevalence of anemia, n (%)	75 (83%)	56 (81%)	19 (91%)	0.32
Chronic anemia, n (%)	55 (76%)[a]	44 (81%)[b]	11 (61%)[c]	0.08
Hemoglobin measurement timepoints (number), median (IQR)	4 (2-8)	4 (2-9)	4 (2-6)	0.64
Anemia Types				
Iron deficiency anemia (IDA)	15 (17%)	12 (17%)	3 (14%)	1.00
Anemia of inflammation (AI)	40 (44%)	30 (43%)	10 (48%)	0.74
Mixed IDA and AI	32 (36%)	23 (33%)	9 (43%)	0.43
Other*	17 (19%)	13 (19%)	4 (19%)	1.00
More than 1 type	19 (21%)	13/54 (24%)	6/18 (33%)	0.44
IF-associated complications				
Liver disease, n (%)	37 (41%)	30 (43%)	7 (33%)	0.41
Chronic kidney disease, n (%)	0 (0%)	0 (0%)	0 (0%)	-
GI bleed, n (%)	22 (24%)	17 (25%)	5 (24%)	1.00
SIBO, n (%)	36 (40%)	30 (43%)	6 (29%)	0.22
Elevated fecal calprotectin	3/20 (15%)	2/15 (13%)	1/5 (20%)	1.00
Endoscopic characteristics				
Endoscopy, n (%)	39 (43%)	27 (39%)	12 (57%)	0.15
Histologic inflammation, n (%)	24 (62%)[d]	16 (59%)[e]	8 (67%)[f]	0.66
Histologic evidence of SIBO, n (%)	18 (46%)[d]	13 (48%)[e]	5 (42%)[f]	0.71
Anastomotic ulcers, n (%)	8 (21%)[d]	7 (26%)[e]	1 (8%)[f]	0.39
Staple line ulcers, n (%)				
Medications/supplementation				
Oral iron supplementation	49 (54%)	38 (55%)	11 (52%)	0.83
Iron supplemented PN	48 (53%)	8 (12%)	2 (10%)	1.00
Intravenous iron treatment	10 (11%)	34 (49%)	13 (62%)	0.31
SIBO antibiotic therapy/prophylaxis	36 (40%)	30 (43%)	6 (29%)	0.22
PPI use	77 (86%)	34 (49%)	13 (62%)	0.31

[#]Other includes those with anemia which did not fit the classification parameters for IDA, AI or Mixed anemia, or, had missing data required to classify the anemia, such as ferritin or sTfR. *Mucosal enteropathy includes microvillus inclusion disease and tufting enteropathy.[a] n=72, [b] n=54, [c] n=18, # of those with anemia and > 1 hemoglobin measurement. [d] n=39, [e] n=27, [f] n=12.
PN = parenteral nutrition; IF = intestinal failure; SBS = short bowel syndrome; STEP= serial transverse enteroplasty; SIBO = small intestine bacterial overgrowth; PPI = proton pump inhibitor.

4.5 Predictors of Anemia

4.5.1 Univariable Regression Analysis

Table 10 shows clinical and demographic predictors of anemia and anemia types used in univariable regression analysis. Odds ratios (OR) express the odds of the outcome of anemia or anemia types compared to no anemia. Odds ratios were unable to be calculated for several variables due to failure to achieve convergence or collinearity. Upon further inspection, it was observed that there were a small number of observations or no observations at all for some of these variables (Table 11), which could contribute to both collinearity and failure to achieve convergence. On univariable analysis, GI bleed and copper deficiency were considered as possible significant predictors of anemia as both had *p*-values < 0.05 and 95% confidence intervals which did not include 1. As expected, children with a history of GI bleed had increased odds of anemia (OR 5.77, 95% CI 1.48-22.43; *p*=0.01) compared to no anemia. Children with copper deficiency had increased odds of anemia (OR 3.65, 95% CI 1.09-12.07; *p*=0.04), as well as mixed IDA/AI (OR 6.26, 95% CI 1.06-36.91; *p*=0.04). Children with IFALD had a decreased odds of mixed IDA/AI (OR 0.20, 95% CI 0.05-0.83; *p*=0.03). Children receiving iron-supplemented PN had a lower odds of mixed IDA/AI compared to anemia (OR 0.06, 95% CI 0.12-0.25; *p*<0.001). Meanwhile, children receiving oral iron supplementation had an increased odds of mixed IDA/AI compared to no anemia (OR 3.40, 95% CI 1.34-8.62; *p*=0.01). An additional analysis to assess the impact of iron-supplemented PN on iron deficiency was performed and the odds of having iron deficiency was lower among children receiving iron-supplemented PN compared to those on PN without iron; OR 0.05 (95% CI 0.02-0.14; *p*<0.001) (not shown in Table 10).

Table 10. Univariable predictors of anemia and anemia types

	Anemia		Iron deficiency anemia (IDA)		Anemia of inflammation (AI)		Mixed IDA/AI	
Predictor	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Demographic characteristics								
Male sex	0.59 (0.29-1.18)	0.14	3.26 (0.45-23.76)	0.24	0.48 (0.17-1.32)	0.15	0.68 (0.19-2.37)	0.55
Age at enrolment	0.94 (0.87-1.02)	0.13	0.23 (0.03-1.52)	0.13	0.78 (0.27-2.29)	0.67	1.83 (0.53-6.43)	0.34
Surgical characteristics								
Short bowel syndrome (SBS)	0.93 (0.40-2.18)	0.87	1.78 (0.19-16.52)	0.61	0.66 (0.20-2.20)	0.49	0.85 (0.19-3.83)	0.84
Ileocecal valve (ICV) present	0.85 (0.42-1.78)	0.69	0.23 (0.03-1.51)	0.13	0.78 (0.27-2.29)	0.66	1.83 (0.52-6.43)	0.34
Serial transverse enteroplasty (STEP)	0.66 (0.28-1.52)	0.32	0.46 (0.06-3.76)	0.47	0.30 (0.08-1.13)	0.08	0.74 (0.17-3.14)	0.68
Intestinal failure (IF) associated complications								
intestinal failure associated liver disease (IFALD)	0.89 (0.45-1.77)	0.74	0.60 (0.12-3.05)	0.54	1.09 (0.38-3.12)	0.87	0.20 (0.05-0.83)	**0.03**
IFALD - any history	1.27 (0.62-2.60)	0.51	2.12 (0.39-11.53)	0.38	1.03 (0.36-2.96)	0.96	0.29 (0.07-1.21)	0.09
Histologic intestinal inflammation	0.97 (0.06-15.94)	0.98	Convergence not achieved		Convergence not achieved		0.29 (0.02-4.00)	0.39
Histologic intestinal Inflammation – any history	0.97 (0.06-15.94)	0.98	Convergence not achieved		Convergence not achieved		0.29 (0.02-4.68)	0.39
GI Bleed	5.77 (1.48-22.43)	**0.01**	5.74 (0.45-73.34)	0.18	1.21 (0.10-14.96)	0.88	2.21 (0.14-35.91)	0.58
GI Bleed - any history	1.20 (0.55-2.64)	0.65	1.96 (0.29-13.25)	0.49	1.29 (0.41-4.10)	0.66	2.14 (0.52-8.75)	0.29
Anastomotic Ulcer	2.82 (0.49-16.13)	0.25	30.74 (0.02-51021)	0.37	2.09 (0.24-18.50)	0.51	11.03 (0.47-256.97)	0.14
Small intestine bacterial overgrowth (SIBO)	1.15 (0.63-2.11)	0.65	1.31 (0.31-5.56)	0.71	0.65 (0.22-1.96)	0.45	1.51 (0.59-3.88)	0.39
High Fecal calprotectin (>150 ug/g)	3.85 (0.12-124.16)	0.45	Convergence not achieved		6.00 (0.67-53.67)	0.11	Convergence not achieved	
Vitamin and micronutrient deficiencies								
Iron deficiency	1.61 (0.85-3.03)	0.14	Collinearity		Collinearity		Collinearity	
Vitamin D insufficiency	1.31 (0.63-2.79)	0.47	1.67 (0.28-10.05)	0.57	0.99 (0.30-3.27)	0.98	1.29 (0.37-3.43)	0.83
Vitamin B12 deficiency	0.32 (0.05-2.16)	0.24	Collinearity		Collinearity		0.35 (0.26-5.98)	0.47
Folate deficiency	Collinearity		Collinearity		Collinearity		Collinearity	
Copper deficiency	3.65 (1.09-12.07)	**0.04**	1.71 (0.06-49.74)	0.75	2.51 (0.32-19.78)	0.38	6.26 (1.06-36.91)	**0.04**
Zinc deficiency	1.10 (0.26-4.60)	0.90	60.92 (0.62-5978.08)	0.08	Collinearity		4.02 (0.55-29.30)	0.17
Low Ceruloplasmin	1.14 (0.21-6.17)	0.88	Collinearity		Collinearity		1.44 (0.12-18.13)	0.78
Medications and supplementation								
Parenteral Nutrition (PN)	2.49 (0.93-6.71)	0.07	3.58 (0.21-61.58)	0.38	3.20 (0.59-17.30)	0.18	1.80 (0.36-9.11)	0.48
Oral iron	1.09 (0.62-1.91)	0.76	1.04 (0.29-3.76)	0.95	0.72 (0.28-1.84)	0.49	3.40 (1.34-8.62)	**0.01**
Intravenous iron	7.40 (1.28-42.88)	**0.03**	12.75 (0.73-221.29)	0.08	14.65 (1.61-133.29)	**0.02**	15.60 (0.99-245.29)	0.05
Iron-supplemented PN	0.80 (0.42-1.53)	0.50	0.55 (0.12-2.42)	0.43	1.59 (0.60-4.19)	0.35	0.06 (0.12-0.25)	**0.0001**
Proton pump inhibitor	1.13 (0.61-2.11)	0.69	1.48 (0.36-6.13)	0.59	1.45 (0.55-3.87)	0.45	0.39 (0.14-1.05)	0.06
SIBO antibiotics	1.07 (0.59-1.95)	0.82	0.70 (0.17-2.90)	0.63	0.35 (0.12-1.05)	0.06	1.33 (0.49-3.61)	0.57

IDA = iron deficiency anemia; AI = anemia of inflammation; IFALD – intestinal failure-associated liver disease; GI = gastrointestinal; PN = parenteral nutrition; SIBO = small intestine bacterial overgrowth

Table 11. Total number of measurements for potential predictor variables

Predictor variable	Total # measurements (448)	Anemia (227)	No anemia (221)	ID (26)	AI (68)	Mixed IDA/AI (58)
Demographic characteristics						
Sex (male)	254/448 (57%)	112/227 (49%)	142/221 (64%)	20/26 (77%)	32/68 (47%)	29/58 (50%)
Age at enrolment	448					
Surgical characteristics						
Short bowel syndrome (SBS)	353/448 (79%)	178/227 (78%)	175/221 (79%)	23/26 (88%)	49/68 (72%)	46/58 (79%)
Ileocecal valve (ICV) present	194/448 (43%)	98/227 (43%)	96/221 (43%)	5/26 (19%)	29/68 (43%)	31/58 (53%)
Serial transverse enteroplasty (STEP)	137/448 (31%)	66/227 (29%)	71/221 (32%)	8/36 (31%)	14/68 (21%)	19/58 (33%)
Intestinal failure (IF) associated complications						
Intestinal failure-associated liver disease (IFALD)	155/445 (35%)	81/227 (36%)	74/218 (34%)	6/26 (23%)	24/68 (35%)	13/58 (22%)
IFALD - any history	197/448 (44%)	110/227 (48%)	87/221 (39%)	16/26 (62%)	28/68 (41%)	19/58 (33%)
Histologic Inflammation	40/51 (78%)	26/33 (79%)	14/18 (78%)	3/3 (100%)	5/5 (100%)	5/12 (58%)
Histologic Inflammation – any history	40/51 (78%)	26/33 (79%)	14/18 (78%)	3/3 (100%)	5/5 (100%)	7/12 (58%)
GI Bleed	20/442 (5%)	16/227 (7%)	4/215 (2%)	2/26 (8%)	2/68 (3%)	2/58 (3%)
GI Bleed - any history	165/448 (37%)	84/227 (37%)	81/221 (37%)	11/26 (42%)	25/68 (37%)	24/58 (41%)
Anastomotic Ulcer	26/91 (29%)	16/50 (32%)	10/41 (24%)	2/6 (33%)	4/14 (29%)	7/12 (58%)
Small intestine bacterial overgrowth (SIBO)	128/428 (30%)	72/216 (33%)	55/206 (27%)	10/26 (39%)	17/65 (26%)	18/57 (32%)
High Fecal calprotectin (>150 ug/g)	7/26 (27%)	4/11 (36%)	3/15 (20%)	0/2 (0%)	3/5 (60%)	1/4 (25%)

*table continued on next page

IDA = iron deficiency anemia; AI = anemia of inflammation

Table 11. Total number of measurements for potential predictor variables…continued

Predictor variable	Total # measurements (448)	Anemia (227)	No anemia (221)	IDA (26)	AI (68)	Mixed IDA/AI (58)
Vitamin and micronutrient deficiencies						
Iron deficiency	166/346 (48%)	100/186 (54%)	66/160 (41%)	26/26 (100%)	0/68 (0%)	57/57 (100%)
Vitamin D insufficiency	72/343 (21%)	38/168 (23%)	34/175 (19%)	6/21 (29%)	8/47 (17%)	12/49 (24%)
Vitamin B12 deficiency	10/353 (3%)	2/173 (1%)	8/180 (4%)	0/21 (0%)	0/58 (0%)	1/48 (2%)
Folate deficiency	2/158 (1%)	0/83 (0%)	2/75 (3%)	0/12 (0%)	0/17 (0%)	0/33 (0%)
Copper deficiency	26/260 (10%)	18/144 (13%)	8/116 (7%)	1/17 (6%)	4/44 (9%)	7/46 (15%)
Zinc deficiency	13/353 (4%)	7/178 (4%)	6/175 (3%)	3/19 (16%)	0/58 (0%)	4/47 (9%)
Low Ceruloplasmin	11/195 (6%)	5/107 (5%)	6/88 (7%)	0/16 (0%)	0/39 (0%)	2/31 (6%)
Medications and supplementation						
PN	409/443 (92%)	102/227 (45%)	193/216 (89%)	25/26 (96%)	65/68 (96%)	54/58 (93%)
Oral iron supplement	182/444 (41%)	101/226 (45%)	80/217 (37%)	11/26 (42%)	24/68 (35%)	38/58 (66%)
Intravenous iron therapy	18/444 (4%)	102/227 (45%)	7/217 (3%)	3/26 (12%)	7/68 (10%)	5/58 (9%)
Iron-supplemented PN	186/442 (42%)	89/225 (40%)	97/217 (45%)	10/26 (39%)	37/67 (55%)	5/53 (9%)
Proton pump inhibitor (PPI)	273/444 (62%)	139/227 (61%)	134/217 (62%)	18/26 (69%)	44/68 (65%)	23/58 (40%)
SIBO antibiotic use	175/435 (40%)	91/218 (42%)	83/211 (38%)	10/26 (39%)	18/66 (27%)	26/58 (45%)

IDA = iron deficiency anemia; AI = anemia of inflammation

5 Chapter 5: Discussion

5.1 Main Findings

Anemia is a frequent complication in children with IF with potentially detrimental consequences. Recent studies have demonstrated that up to 50% of children with IF have motor and cognitive delay and school-aged children have significantly lower health related quality of life compared to both healthy children and children with other chronic diseases.[165–167] Thus, anemia is a potentially treatable and preventable contributor to neurodevelopmental delay and poor quality of life in children with IF. Increasing awareness and concern regarding the high prevalence of iron deficiency and IDA in children with IF has prompted the development of recommendations regarding monitoring and treatment of iron deficiency.[29] However, recent studies have reported that in addition to IDA, AI may be equally important.[59] The contributions of the various types of anemia in IF, namely IDA, AI and mixed IDA/AI has, as far as we are aware, not previously been explored and subsequently, the underlying causes of these anemias is unknown. An improved understanding of anemia in children with IF will enable clinicians to more effectively differentiate, treat, and even prevent anemia and will contribute to improved long-term outcomes and quality of life for these children.

In this study we set out to determine the 10-year period prevalence of anemia, as well as the prevalence of anemia types, namely IDA, AI and mixed IDA/AI, in children with IF and to identify potential factors associated with these types of anemia. This study represents the first study of anemia in children with IF in Canada and is unique in that it describes the chronic burden of anemia over time in these children. The results of our study demonstrated that 1) the overall

prevalence of anemia was high in children with IF; 2) this anemia was often chronic over several years; 3) both AI and mixed IDA/AI occurred more frequently than IDA; and 4) children frequently developed more than one type of anemia over time. While the precise underlying causes of these anemias remain unknown, our study identified associations which support proposed theories that SIBO, intestinal inflammation and liver disease may be drivers of inflammation which contribute to anemia in these children. Furthermore, the opportunity to compare differences in anemia types and frequencies of IF-associated complications between children who routinely receive iron-supplemented PN and those who receive PN without iron revealed that the mode of administration of iron supplementation may impact development of various types of anemia and IF-associated complications. In our study, children receiving iron-supplemented PN had a lower odds of developing mixed IDA/AI, a lower frequency of SIBO and GI bleeds, and a higher frequency of IFALD.

5.1.1 Prevalence of Anemia

The period prevalence of anemia in children with IF in our study was 83%, which is in keeping with previous reports from single center retrospective studies which reported the prevalence of anemia ~ 90%.[55–59] Among children with anemia who had $\geq$ 2 hemoglobin measurement timepoints during the study, 76% had chronic and/or recurrent anemia. The prevalence of chronic anemia was higher in children followed by the 2 IRPs in Alberta (90%) compared to Toronto (66%), however, this difference may be due in part to the overall shorter observation period in Toronto compared to Alberta and as a result, the lower number of hemoglobin measurement timepoints in Toronto. Given that every single hemoglobin

measurement for each patient over the study period was not collected, we were unable to differentiate between chronic anemia that was truly chronic and unremitting, versus recurrent anemia that briefly resolved and recurred. The heat map (Figure 8) depicting anemia status and anemia type at each hemoglobin measurement timepoint for each child does indeed provide evidence that some children experienced recurrent anemia as opposed to chronic, unremitting and/or treatment refractory anemia, as evidenced by interval years of no anemia interspersed between years with anemia. Perhaps more intriguing is the evolution of different anemia types in individuals over time as well as the persistence of anemia despite oral or PN iron supplementation. The depiction of not only recurrent anemia, but also persistent and changing anemia types over many years suggests that the underlying pathophysiology of anemia is a complex and dynamic process in children with IF. In addition, the persistence of anemia despite chronic oral iron supplementation highlights the need for a prospective study examining the dosing and efficacy of oral vs IV vs PN iron supplementation on not only anemia, but other potential contributors to anemia, such as changes in the intestinal microbiome, intestinal inflammation, and liver disease, all of which could be contributing to the development, persistence, and evolution of anemia types.

5.1.2 Types of Anemia

Describing the prevalence of the types of anemia in children with IF is an important first step in understanding the underlying etiologies of these anemias and developing an approach to effective management and prevention of these anemias. Surprisingly, while iron deficiency was common, affecting 61% of children with IF in our study, the period prevalence of IDA was relatively

low at 17% compared to the prevalence of mixed IDA/AI (36%) and AI (44%). The high rates of AI and mixed IDA/AI was striking. Given that we did not include measures of anemia which occurred during episodes of acute illness or inflammation, including central line associated bloodstream infections and sepsis, urinary tract infections, respiratory infections which were positive by molecular techniques for common viral infections such as influenza, COVID or entero-rhinovirus, or within 3 months of a major surgical procedure, these results suggest a high burden of chronic and/or subclinical inflammation in these children. Concerns around anemia in children with IF and other patient populations on long term home PN have largely focused on the burden of IDA. The realization that a large proportion of anemia in children with IF may have an inflammatory component may explain why some children have persistent anemia that is refractory to treatment with iron supplementation and also why the type of anemia can change over time. A recent retrospective study by Namjoshi et al. (2020) examining iron status in a population of children and adults with IF on PN support our findings as 36% of measurements of hemoglobin and iron indices in their study were consistent with a diagnosis of AI; an obvious cause of the inflammation in their patient population was not identified. [59]

5.1.3 Potential Contributors to Inflammation in Anemia

Potential underlying contributors to chronic inflammation resulting in AI and mixed IDA/AI include CKD, IFALD, chronic intestinal mucosal inflammation, SIBO and the provision of PN itself. Namjoshi et al. (2020) found a high prevalence of CKD stage 2 or 3 in their study and CKD was correlated with AI; none of the children in our study had CKD.[59] IFALD was present in 41% of children with IF in our study, but it was not found to be a risk factor for AI or mixed AI/IDA on

univariable regression analysis. Interestingly, children with IFALD had a lower odds of having mixed IDA/AI (OR 0.20, 95% CI 0.05-0.83; *p*=0.03); this requires further exploration as it is incongruent with biologic plausibility. It is likely that this result may be an artefact of the low prevalence of mixed IDA/AI in Toronto (9%) compared to Alberta (75%) (*p* <0.001) combined with the significantly increased prevalence of IFALD in Toronto (52%) compared to Alberta (25%) (*p*=0.01). The odds ratios evaluating the risk of IFALD in anemia and other anemia types did not approach statistical significance; all had large p-values and 95% CIs that crossed 1.

There is increasing evidence of chronic, intestinal mucosal inflammation in some children with IF, and it has been hypothesized that intestinal dysbiosis and/or SIBO are contributes to intestinal inflammation[69,71,72,74,90,91,93,94,99] Similar to the AI and mixed IDA/AI seen in children with IBD, it is reasonable to propose that chronic intestinal inflammation and immune system activation may be the source of inflammation in AI and mixed IDA/AI in children with IF. We assessed for histologic intestinal mucosal inflammation in 39 patients who underwent endoscopy during the study period and histologic inflammation was present in 24 (61%) of these children. A recent case control study by Culbreath et al. (2023) revealed that 101/365 (28%) of children with IF followed at Boston Children's Hospital over an 11-year period had inflammation on intestinal biopsies and thus, the prevalence of intestinal inflammation in the children in our study is high in comparison.[94] Unfortunately, routine endoscopy is not standard of practice at the IRP centers in our study. Thus, nearly all children who underwent endoscopy in our study did so to evaluate for causes of anemia or GI bleeding and thus, the frequency of intestinal inflammation may have been skewed higher in those children with anemia and thus, may not be reflective of the true prevalence of intestinal inflammation in our patient population. While there was no statistically

significant difference in the frequency of histologic inflammation between those with and without anemia in our study, only 3 children without any anemia during the study period underwent endoscopy. Sparse data like that observed with endoscopy threatens internal validity and thus, while histologic intestinal mucosal inflammation failed to be a statistically significant predictor of not only AI but anemia itself in our study, it may potentially be shown to be significant when evaluated in a cohort of children with IF who undergo routine endoscopy. The increased prevalence of anemia and specifically mixed IDA/AI in children with IF who have a history of GI bleeding combined with the increased prevalence of histologic inflammation and SIBO in children with a history of GI bleeding does suggest that intestinal inflammation is important in inflammation associated with anemia.

SIBO is a frequent complication in children with IF and has been associated with both local and systemic inflammation.[74,99,168] SIBO can contribute to intestinal mucosal erosions and ulcers along with villous blunting, increased lymphocytic infiltration and even crypt architectural changes on histology.[151,152] Indeed, such changes were observed in in 46% of children in our study who underwent endoscopy. A small pilot study examining SIBO in children with SBS found increased serum concentrations of TNF-α in children with SIBO compared to those without SIBO, while a more recent study of SIBO in adults without IF found increased levels of IL-1β, TNF-α and IL-6 in duodenal aspirates in those with SIBO compared to those without SIBO.[74,169] These studies suggest that a systemic immune response to SIBO exists and could contribute to AI. Unfortunately, despite a high prevalence of SIBO in our study, SIBO was not found to be a risk factor for anemia on regression analysis. SIBO is largely a clinical diagnosis in practice and the symptoms overlap considerably with symptoms related to SBS and gut dysmotility which is

inherent in this population, and thus, it is possible that the true prevalence of SIBO was inflated in our study as the majority of cases had no accompanying objective criteria to confirm or support the diagnosis, such as cultures or histology, and classification was dependent on clinical details obtained from clinic notes in the EMR at the time of the hemoglobin measurement. There is a growing interest in understanding the alterations in the gut microbiome in children with SBS and IF. Several studies to date have demonstrated reduced microbial diversity in children with SBS on PN with an increased abundance of pro-inflammatory bacterial species such as Enterobacteriaceae.[97,98] Indeed, decreased microbial diversity and overgrowth of certain species has also been shown to be associated with IFALD and systemic inflammation in patient populations with chronic liver disease.[101,168] Future studies examining more nuanced changes in abundance of specific bacterial species or microbial metabolites in children with IF with and without anemia may help to further elucidate the role of SIBO and alterations in the gut microbiome in anemia in children with IF.

The provision of PN itself can promote both systemic and organ specific inflammation. The role of PN in promoting cholestasis and liver injury is well established and there is increasing evidence supporting increased levels of inflammatory cytokine levels and T-cell dysfunction in both animal models and patients on long-term PN which could contribute to the maintenance of a chronic inflammatory response.[89,170,171] This relationship between PN use and inflammation is further complicated by the impact of the lack of enteral nutrition associated with PN use and resulting alterations in the gut microbiota on inflammation. For example, Cole et al. (2010) demonstrated an inverse relationship between serum concentrations of pro-inflammatory cytokines TNF-α, interleukin-1 beta (IL-1β), IL-6 and IL-8 and percent calories enterally fed to

children with IF and SBS;[57] meanwhile a retrospective study of 57 children with IF found that children weaned from PN were less likely to have SIBO and Kaufman et al. (1997) found that intestinal inflammation was associated with longer duration of PN and was more severe in children who were PN dependent compared to those who were weaned.[78,99] While the frequency of anemia in our study was higher when children were on PN vs off PN (52% vs 32%, p=0.03), only 34 of 448 lab measurements were obtained in children who were off PN at the time of their lab measurements and PN use was not statistically significant on univariable regression analysis. Our study was not designed to compare differences in prevalence of anemia in children on PN vs off PN, and while we had hoped to examine the impact of percent PN dependence (as defined by the ratio of non-protein energy intake over resting energy expenditure) on anemia, it was ultimately not practical nor feasible to assess percent PN dependence in every child at every timepoint from file review across 3 centers.

In summary, our study was not able to identify, with statistical confidence, specific risk factors or sources of chronic inflammation or immune activation involved in AI and mixed IDA/AI. Sparse and missing data, as in the case of limited histologic data, FCP measurements and objective confirmation of a diagnosis of SIBO, limited our ability to detect statistically significant predictors despite their clinically relevant associations. This was also complicated by the lack of an objective and reliable marker of inflammation in this study and this patient population. CRP and ESR were not routinely elevated in cases of anemia that clearly fit a definition of AI or mixed IDA/AI based on MCV, ferritin, TSAT and/or sTfR levels. There is a subset of individuals with inflammatory bowel disease who fail to mount a CRP response with active intestinal disease, and Moran-Lev et al. (2023) similarly found that CRP was normal in children with IF with chronic intestinal mucosal

inflammation.[159,160] Thus, while an elevated CRP is helpful in interpreting iron studies and classifying anemia types, a normal CRP level does not rule out the possibility of chronic, subclinical inflammation which may contribute to AI or mixed IDA/AI. There are mixed results in the literature around the utility of FCP in detecting intestinal inflammation and/or SIBO in children with IF; Cole et al. (2010) reported increased levels of FCP in children with IF with SIBO compared to those without SIBO, while other studies looking for non-invasive markers of SIBO in the general population report no difference in FCP levels in those with and without SIBO.[162] We did not have sufficient samples of FCP in our study to assess the reliability of FCP in our patient population. Looking at serum markers of inflammation, such as TNF-α, IL-6 or IL-8 may be helpful in interpreting iron status and inflammation in anemia, however, this has not been explored extensively, particularly in the pediatric IF population. Serum hepcidin is another potentially useful marker to identify the presence of inflammation, but it is not widely available and is used mainly in the research setting at this time.[25,29] Finally, it is likely that multiple etiologies for inflammation coexist in these children and interact in complex and dynamic ways and understanding these relationships in a highly heterogenous patient population will require well designed studies across intestinal rehabilitation programs and centers to accomplish this end.

5.1.4 Vitamin and Micronutrient Deficiencies

Several studies have reported a high prevalence of vitamin and micronutrient deficiencies in children with IF.[55–58] Interestingly, the prevalence of copper (7%) and zinc (10%) deficiency were lower in our study in comparison to those previously reported which have reported the prevalence of copper ranging between 22-56% and zinc deficiency between 20-

52% in children with IF on PN.[55–58] We did not consider low levels of copper or zinc as deficient in the presence of an elevated CRP, as these micronutrients have been reported to be depressed during acute phase response[118,144] Only one study reported taking CRP into consideration in their assessment of zinc and copper deficiency, however, the frequencies of deficiency in our study were still lower in comparison to that study. While there is evidence in the literature the deficiencies in zinc and copper can contribute to the development and persistence of anemia in other patient populations, there is a lack of research on the impact of vitamin and micronutrient deficiencies on anemia in children with IF.[106,107] Namjoshi et al. (2018) reported that zinc deficiency and multiple micronutrient deficiencies were associated with severe anemia (defined as hemoglobin <8.5 g/dL) and copper and iron deficiency showed a trend towards an association with severe anemia.[57] Copper deficiency, but not zinc deficiency, was found to be associated with an increased odds of both anemia (OR 3.65, 95% CI 1.09-12.07; *p*=0.04) and mixed IDA/AI (OR 6.26, 95% CI 1.06-36.91; *p*=0.04) on univariable analysis in our study. However, these findings from the univariable regression analysis relating to copper deficiency and anemia should be interpreted with caution based on the relatively wide CIs and given that all micronutrient levels were not always collected at the same time as the hemoglobin measurements.

There is some variation in the literature regarding cut-offs for vitamin D insufficiency and deficiency, which could be due in part to evolving definitions over the last few years, making direct comparisons with previous studies difficult. The threshold for vitamin D deficiency used in our study (<25 nmol/L) was lower than the threshold used in previous studies; Namjoshi et al. (2018) defined vitamin D deficiency at <30 ng/mL (equivalent to 75 nmol/L), Ubesie et al. (2013) defined

vitamin D deficiency at <20 ng/mL (equivalent to 50 nmol/L) and insufficiency as 20-30 ng/mL (equivalent to 50-75 nmol/L) while Yang et al. (2011) defined deficiency at <30 ng/mL (equivalent to 75 nmol/L).[55–57] Our threshold for vitamin D insufficiency (25-50 nmol/L) is most in keeping with the definition of deficiency used in other studies. With that in mind, the prevalence of vitamin D insufficiency in our study (52%) was lower to the prevalence of "deficiency" reported by Namjoshi et al. (2018) (67%), but higher than that reported by Ubesie et al. (2013) (34.5%).[56,57] Vitamin D has been found to play a role in downregulating pro-inflammatory cytokines (IL-6 and IL-1B) as well as hepcidin and thus impact iron dynamics in anemia. There were no children with vitamin D deficiency in our study and while 52% met criteria for vitamin D insufficiency, insufficiency was not predictive of anemia on univariable regression analysis.

Iron deficiency was highly prevalent in our study and interestingly, was not found to be a statistically significant predictor of anemia on regression analysis, as was similarly reported in a previous study by Namjoshi et al. (2018).[57] Given the high rates of oral iron supplementation seen in the absence of anemia in Figure 8, it is possible that clinicians were screening for and identifying iron deficiency in its early stages and initiating iron supplementation to prevent evolution to anemia. Odds ratios were unable to be calculated for iron deficiency with each of the anemia types due to collinearity. On review of the measurement counts in Table 11, it is evident that this collinearity occurred because all the cases of IDA and mixed IDA/AI also had iron deficiency while none of the cases of AI had iron deficiency.

5.1.5 Mechanism of Iron Supplementation

This study presented an opportunity to compare prevalence of anemia as well as IF associated complications between intestinal rehabilitation centres that provide iron-supplemented PN versus those that do not. Our study identified several differences between children with IF followed in Toronto compared to Alberta and highlight some intriguing questions around the mode of iron supplementation which require further investigation. First, the prevalence of iron deficiency was lower on both an individual and event-level basis in Toronto where iron-supplemented PN was routinely used in comparison to Alberta where iron-supplemented PN was not available. Regression analysis revealed that the use of iron-supplemented PN compared to PN without iron supplementation was associated with a statistically significant lower odds of developing iron deficiency (OR 0.05, 95% CI 0.02-0.14; $p<0.01$). Certainly, the provision of iron in PN provides a more preventative approach to iron deficiency and potentially more effective treatment in comparison to oral iron supplementation; it is easier to adjust dosing of iron in the PN all the while avoiding the potential shortcomings of oral iron supplementation which include poor enteral tolerance, potentially poor absorption in the presence of intestinal inflammation and poor adherence with oral iron supplementation. Second, while the period prevalence of IDA on a patient-level basis was not different between the two IRP locations, the prevalence of mixed IDA/AI was lower in Toronto (9% in Toronto vs 75% in Alberta, $p<0.001$), and the use of iron-supplemented PN was associated with a decreased odds of developing mixed IDA/AI compared to no anemia (OR 0.06, 95% CI 0.12-0.25, $p=0.0001$). This finding is intriguing for several reasons. First, there is a theoretical concern that enteral iron supplementation can contribute to dysbiosis. This is important when we consider that Alberta,

which had a higher prevalence of oral iron supplementation also had a higher prevalence of SIBO and GI bleeding, and GI bleeding was associated with a higher prevalence of histologic evidence of intestinal inflammation in our study. Certainly, the inflammation created by dysbiosis and SIBO could drive the inflammatory component of mixed IDA/AI, while the intestinal inflammation could in turn impair oral absorption of iron and contribute to the intestinal mucosal erosions/bleeding, thus driving the iron deficiency component of mixed IDA/AI. The second major reason this finding is intriguing is there was a trend towards a higher prevalence of AI in Toronto compared to Alberta. Not only were ferritin levels higher in Toronto across those children with and without anemia, but the prevalence of IFALD was also higher in Toronto. These are important findings given the concern that iron in PN can contribute to formation of reactive oxidants and increase the risk of iron overload resulting in liver injury.[172] Our study was not positioned to evaluate a causal relationship between IFALD and iron inclusive PN, but it raises the possibility that while iron supplemented PN in Toronto was protective against mixed IDA/AI because it reduced the need for oral iron supplementation which drives both the inflammatory and iron deficiency processes in mixed IDA/AI, iron supplemented PN may drive inflammation through increased liver injury and thus, result in an increased frequency of AI as opposed to mixed IDA/AI. This is an area which warrants future study and is of clinical importance when considering long-term safety and potential negative consequences of using iron-supplemented PN.

5.1.6 Regression Analysis

Univariable regression analysis was performed to further examine the relationships between exposure variables and anemia and anemia types with the aim of identifying and

assessing the strength of potential associations. The regression analysis revealed that children who had GI bleed had increased odds of developing anemia (OR 5.77, 95% CI 1.48-22.43; *p*=0.01). GI bleeding is an exposure variable that is both clinically relevant and certainly has biological plausibility. GI bleeding was not predictive of any of the anemia types specifically; the ORs were >1 for each anemia type, but the p-values were all >0.5 and 95% CIs crossed 1. Surprisingly, history of STEP and presence of AU, both of which have been observed to be sources of GI bleeding in children with IF were not predictive of anemia.[79,82,83,173] Certainly, the presence of unidentified AU and mild or occult GI bleeding due to the lack of endoscopic assessment for all participants may explain, in part, this lack of association between AU and anemia.

Univariable regression also identified several other potential predictor variables for anemia and anemia types that have biological plausibility, including copper deficiency, iron-supplemented PN and oral iron supplementation, as discussed in sections 5.1.4 and 5.1.5. Intravenous iron supplementation was associated with an increased odds of both anemia (OR 7.40, 95% CI 1.28-42.88; *p*=0.03) and AI (OR 14.65, 95% CI 1.61-133.29; *p*=0.02). However, given that IV iron is administered in response to iron deficiency and anemia, this finding is not of any clinical significance. IFALD was identified as a potential predictor protective for mixed IDA/AI (OR 0.20, 95% CI 0.05-0.83, *p*=0.03), but IFALD lacks biological plausibility as being protective for mixed IDA/AI or any anemia type. It is reasonable to suspect that there may be confounding factors impacting this variable, for example the use of PN that is not supplemented with iron, which, if included in a multivariable model of regression analysis, would no longer be statistically significant.

Given the large number of variables being examined in this exploratory study, along with the quantity of missing values/information and a relatively small sample size, multivariable regression analysis was not performed due to the risks of overfitting and multicollinearity. Indeed, the presence of collinearity and small numbers was evident for numerous variables on univariable regression alone (Table 10, Table 11). In addition, the missing data makes it difficult to accurately identify possible confounding variables or effect modifiers and determine which variables to include in the regression model and thus, could introduce bias and reduce the validity of the model and lead to potentially unreliable conclusions.

The pediatric IF population is a small and heterogeneous population with multiorgan pathology. As we enter a new phase of IF management where children are surviving into adulthood clinicians are increasingly faced with the discovery of new complications in these children and it is becoming apparent that the relationships between PN, EN (or absence/delay of EN), altered intestinal motility, chronic and repeated exposure to antibiotics, the intestinal microbiome and local and systemic inflammation are incredibly complex and dynamic. With so many variables, complex relationships and potential feedback loops, regression analysis may not be the best tool to try to understand how all of these factors relate to anemia. Directed acyclic graphs (DAGs) provide a means of graphically representing causal relationships between variables and are helpful in delineating confounding and mediating variables and potential sources of bias in exposure-outcome relationships.[174,175] DAGs are useful tools when the underlying mechanisms are complex and regression analysis is not suitable. While building a DAG will require making some assumptions about relationships between variables, these assumptions are made explicit in the DAG and are useful in communicating these assumptions

in a transparent way.[176] While DAGs are essentially qualitative in nature, they can help to identify variables to include in regression models to reduce confounding and obtain an estimate of causal effects. For future research, DAGs may prove to be a useful tool in helping to visualize and understand the possible relationships between route of iron supplementation (PO vs IV vs PN), identified as possible predictor variables in our univariable regression, with intestinal inflammation, GI bleeding, liver disease and anemia and anemia types in future studies.

5.2 Limitations

There were several limitations to this study. The first limitation was the small sample size. Small sample size is a common struggle in studies in children with IF as it is a rare disease. The multicenter study design enabled us to increase our study size to 90 participants, which is considerably large in comparison to most other studies in this patient population. However, when looking at multiple outcomes (anemia plus the 3 anemia types), a small sample size results in low cell counts and limits the power to be able to identify potential predictor variables. Study power was further reduced due to the large number of potential predictor variables we examined in this exploratory study.

A major limitation of our study was the retrospective study design, which resulted in missing and sparse data. Sparse data was clearly apparent for endoscopy, histology and FCP, among other variables, and further contributed to the lack of power to determine the potential role of these variables in anemia and anemia types. The study design also resulted in a lack of ability to temporally relate vitamin and micronutrient deficiencies, as well as endoscopic and histologic findings with anemia and anemia types.

There were several potential sources of misclassification bias in our study with regards to the classification of the types of anemia. The first potential source of misclassification bias came from the difference in laboratory investigations available between Alberta and Toronto to classify the anemia types (e.g. TSAT and ferritin vs sTfR). The second potential source of misclassification bias was the secondary anemia classification scheme that was developed in response to the discovery that the majority of anemia measurements did not fit the primary classification scheme. This secondary anemia classification scheme relied on the assumption that common markers of inflammation, such as CRP and ESR, are not valid markers of inflammation in the pediatric IF population and this secondary classification scheme has not been externally validated. Another limitation was that we did not evaluate the impact of the dose of iron used in oral or PN supplementation on anemia, anemia types or ferritin levels. Additionally, missing TSAT measures from patients in Toronto prevented us from evaluating for evidence of iron overload when measures of ferritin were elevated.

5.3 Implications and Knowledge Translation

This is the only study to date to evaluate the prevalence of the various types of anemia in children with IF. It includes 90 children cared for by three intestinal rehabilitation centers across Canada with subtle, yet important differences in management, specifically the use of iron supplemented PN which allowed for a sub-analysis to evaluate the impact of this difference on the frequencies of anemia and its types. While this study is exploratory and there are numerous limitations as described above, the relatively large sample size for a study in this patient population and multicentre design enhance generalizability. Thus, the prevalence of anemia and

rates of IF-related complications such as SIBO, IFALD an intestinal inflammation, can be considered to be representative of the pediatric IF population as a whole across Canada. The variability in nutritional support, antibiotic use and approaches to management by intestinal rehabilitation programs around the world, may limit the generalizability of these results outside of Canada.

The results of this thesis work will impact clinical decision-making, guideline development and the children with IF and their families. Over the course of this thesis study there has been growing concern surrounding the prevalence of anemia and iron deficiency among clinicians caring for children with IF. This led to publication of a position paper on the evaluation and management or iron deficiency from the NASPGHAN Intestinal Rehabilitation Special Interest Group.[29] The results of this thesis study provide a foundation of knowledge from which we can begin to address some of the research gaps highlighted in the position paper, including the risks and benefits of iron supplemented PN and the potential role of intestinal inflammation and SIBO in anemia. Furthermore, this thesis study draws attention to the high rates of AI and mixed IDA/AI in children with IF, as opposed to isolated IDA, and will help to direct research on understanding the underlying physiology and contributors to AI in these children.

The pediatric IF community is small and clinicians and researchers rely on shared experiences to guide management. The results of this study will be presented next month at the Pediatric Intestinal Failure and Rehabilitation Symposium (PIFRS) in Pittsburgh, PA and preliminary results were previously shared at the Congress of the Intestinal Rehabilitation and Transplant Association (CIRTA) in Chicago, IL in 2023. These biannual conferences are the two major international IF meetings and provide a forum for physicians, surgeons, dietitians and social

workers who make up the intestinal rehabilitation teams to share information and establish multicenter collaborations needed to advance guideline development and clinical care. The results shared at PIFRS will also be published in a special edition of the journal “Intestinal Failure” in the coming months. In addition, I have been invited by the GIFT team at Toronto SickKids Hospital to formally present the study results in October 2024 and discuss some additional sub-analyses to help guide their decision around whether or not to continue the use of iron-supplemented PN in children with IF at their center. Thus, these study results may have a direct impact on the management of anemia in one of the centers involved in this study in the very near future.

Finally, as it is ultimately the children with IF and their families who experience anemia and will benefit from our research findings, we have several initiatives underway to help share our study results and also understand the lived experiences of these children with anemia. In addition to a formal presentation of our study results at our annual IF Family Day, we are currently working on producing an electronic resource for families so they can directly access the results of this study and others currently underway. We are also working to establish a formal partnership with patients/families to share their experiences through digital storytelling. This will provide our IF families with a means by which they can communicate their lived experiences with us and help us as clinicians better understand the impact of anemia and our approach to management on their lives and ultimately promote a shared model of care with families.

5.4 Future Directions

The overwhelming prevalence of anemia in children with IF, its persistence over time and potential long-term consequences highlights the need for an organized approach to diagnosis and management. In addition, a more solid understanding of the underlying contributors to anemia and the relationships between these contributors is needed to achieve this end. A reliable indicator of iron and inflammation status is desperately needed. Future studies evaluating the utility of hepcidin and other non-invasive pro-inflammatory markers of inflammation such as TNF-α, IL-6 or IL-8 are needed, as well as prospective studies which can further delineate the utility of using FCP as a non-invasive tool to assess for intestinal inflammation, SIBO and dysbiosis-associated anemia in the pediatric IF population. There is ongoing discussion around developing an international multi-site registry for the pediatric IF population. This would enable prospective randomized comparative assessments of the efficacy and safety of different iron supplementation modalities and facilitate collaboration for larger, multicenter prospective studies evaluating the role of the gut microbiome and dysbiosis in SIBO, intestinal inflammation, liver disease and anemia. Similar to the growing interest in the role of the diet on the intestinal microbiome and intestinal inflammation, there is growing interest regarding the interplay of the diet, gut microbiome and inflammation in children with IF and this is currently an area in which several IF clinicians and researchers in Canada are actively working to develop. A better understanding of this relationship not only holds promise in improving our understanding of the dynamics of iron and inflammation in anemia, but also holds promise for identifying approaches to prevention of anemia in these complex and fragile children.

5.5 Conclusions

This thesis study represents the first study to assess the prevalence of IDA, AI and mixed IDA/AI in the pediatric IF population. Children with IF are medically complex and there is extensive heterogeneity between children with IF due to their altered anatomy and surgical history and the resulting complications including changes in intestinal perfusion, motility and intestinal microbiota, as well as variability in oral and enteral feeding tolerance, antibiotic exposure and infection history, concomitant liver disease and history of prematurity and related comorbidities. This study highlights the challenges in identifying predictors of anemia and anemia types in children with IF as multiple etiologies may coexist and available laboratory assessment paradigms to classify anemia types are flawed. Further research utilizing multi-site registries for children with IF could help to assess the impact of the different modalities of treatment and prevention of anemia (*e.g.* oral vs IV vs PN iron supplementation, prophylactic antibiotics for SIBO) in this complex patient population and drive improvement in management. Registries could also serve to evaluate the precise impact of the high prevalence and persistence of anemia in children with IF identified in this study on the health, development, and long-term outcomes in these vulnerable children. The hope is that this thesis has helped to contribute to the body of knowledge regarding the burden of anemia in pediatric IF and draws attention to the need for further multicenter research to gain insight into the underlying physiology driving the various types of anemia required to develop an evidence-based approach to diagnosis, management and prevention of anemia in these medically complex and vulnerable children.

6 References

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7 Appendices

Table 1-1: Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) Statement - Checklist of items that should be included in reports of cohort studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	i
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	i
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	Literature Review – Chapter 1, page 1-25. Rationale – Chapter 2, page 26
Objectives	3	State specific objectives, including any prespecified hypotheses	Chapter 2.2, page 26-27
Methods			
Study design	4	Present key elements of study design early in the paper	Chapter 3.1, page 28-30
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Chapter 3, page 28-44
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Chapter 3.1, page 28-30
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Chapters 3.3 and 3.4, page 37-44 Appendix Tables 2-1, 3-1 and 4-1

Table 1-1: Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) Statement - Checklist of items that should be included in reports of cohort studies …continued

	Item No	Recommendation	Page number
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Chapters 3.2 - 3.4, page 30-44
Bias	9	Describe any efforts to address potential sources of bias	Chapter 3.1, page 29
Study size	10	Explain how the study size was arrived at	Chapter 3.1, page 28-30
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Chapter 3.4, page 40-44 Chapter 3.6, page 45-47 Appendix Table 4-1
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Chapter 3.6, page 45-47
		(*b*) Describe any methods used to examine subgroups and interactions	Chapter 3.6, page 46
		(*c*) Explain how missing data were addressed	Chapter 3.4, page 40-44 (missing data and anemia classification)
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	n/a

Table 1-1: Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) Statement - Checklist of items that should be included in reports of cohort studies …continued

	Item No	Recommendation	Page number
Results			
Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Chapter 3.1, page 30 Chapter 4.1.1, page 48
		(b) Give reasons for non-participation at each stage	Reason for parental refusal of consent not provided
		(c) Consider use of a flow diagram	Not done
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Chapter 4.1, page 48-53
		(b) Indicate number of participants with missing data for each variable of interest	Missing annual hemoglobin measurement timepoints Chapter 3.2, page 33 Chapter 4.2.1, page 54-56 Figure 6, page 61-62
		(c) Summarise follow-up time (eg, average and total amount)	Chapter 4.1.1, page 48-49 Chapter 4.1.3, page 52
Outcome data	15	Report numbers of outcome events or summary measures over time	Chapter 4.2, page 54-62 Chapter 4.3, page 63-64

Table 1-1: Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) Statement - Checklist of items that should be included in reports of cohort studies …continued

	Item No	Recommendation	Page number
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Chapter 4.5.1, page 80-83
		(*b*) Report category boundaries when continuous variables were categorized	Chapter 3.3, pages 37-40 Appendix Table 2-1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Chapter 4.4, page 65-79
Discussion			
Key results	18	Summarise key results with reference to study objectives	Chapter 5.1; Primary/secondary objectives Chapter 5.1.1 and 5.1.2, page 84-87 Exploratory objectives Chapter 5.1.3-5.1.6, page 87-99
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Chapter 5.2, page 99-100

Table 1-1: Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) Statement - Checklist of items that should be included in reports of cohort studies …continued

	Item No	Recommendation	Page number
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Chapter 5.1, page 84-99
Generalisability	21	Discuss the generalisability (external validity) of the study results	Chapter 5.3, page 100-102
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Chapter 3.1, page 30

Table 2-1: Normal reference ranges for variables being investigated in serum and stool

Variable	Normal Reference Range
Hematologic indices	
Hemoglobin	
Age 6 months – 2 years	106-145 g/L
Age 3 – 11 years	110-157 g/L
Male 12-14 years	125-170 g/L
Female ≥ 12 years	120-160 g/L
Males 15 ≥ years	137-180 g/L
Mean corpuscular volume (MCV)	
Age 6 months – 2 years	71-90 fL
Age 3 – 11 years	75-91 fL
Age ≥ 12 years	82-100 fL
Mean Corpuscular Hemoglobin Concentration (MCHC)	
Age 6 months – 2 years	310-350 g/L
Age 3 – 11 years	315-360 g/L
Age ≥ 12 years	320-360 g/L
Red cell distribution width (RDW)	11-16 %
Platelets	150-400 $x10^9$/L
White blood cell count (WBC)	
Age 6 months – 2 years	6.0-16.0 $x10^9$/L
Age 3 – 11 years	4.0-14.0 $x10^9$/L
Age ≥ 12 years	4.0-11.0 $x10^9$/L
Reticulocyte	0.2-2.0 %

*table continued on next page

Table 2-1: Normal reference ranges for variables being investigated in serum and stool…continued

Variable	Normal Reference Range
Iron indices	
Iron	8-35 μmol/L
Ferritin	
Age 6 months – 15 years	15-100 μg/L
Female > 15 years	20-300 μg/L
Male > 15 years	30-500 μg/L
Transferrin Saturation (TSAT)	0.15-0.50 %
Total iron binding capacity (TIBC)	50-85 μmol/L
Soluble transferrin receptor (sTfR) (reference values for Toronto Sick Kids Hospital Laboratory)	
1 - < 12 years	0.8-1.6 mg/L
12 - < 19 years	0.7-1.5 mg/L
sTfR-Ferritin index	
1-11 years	0.49-1.5
12-18 years	0.51-1.5
Micronutrients and vitamins	
Vitamin A	0.9-1.7 μmol/L
Vitamin D (25-OH vitamin D)	50-125 nmol/L
Vitamin E	10-21 μmol/L
Copper	11.0-28.0 μmol/L
Ceruloplasmin	0.16-0.45 g/L (female) 0.15-0.30 g/L (male)
Zinc	8-20 μmol/L
Vitamin B12	155-700 pmol/L
Folate	> 12.1 nmol/L
Methylmalonic acid (MMA)	0-0.4 μmol/L

*table continued on next page

Table 2-1: Normal reference ranges for variables being investigated in serum and stool…continued

Variable	Normal Reference Range
Markers of inflammation	
Albumin	
< 1 year	22-45 g/L
≥ 1 year	30-45 g/L
C-reactive protein (CRP)	< 8.0 mg/L
Erythrocyte sedimentation rate (ESR)	
0-12 years	0-10 mm/hr
> 12 years	0-20 mm/hr (female) 0-15 mm/hr (male)
Fecal calprotectin (FCP) (stool test)	< 150 mg/Kg
Markers of liver disease	
Bilirubin – direct	0-7 μmol/L
Alanine aminotransferase (ALT)	< 35 U/L
Aspartate transaminase (AST)	
< 1 year	10-65 U/L
1-3 years	10-55 U/L
4-10 years	10-45 U/L
≥ 11 years	8-32 U/L (female) 8-40 U/L (male)
Alkaline Phosphatase (ALP)	
< 1 year	130-500 U/L
1-12 years	130-430 U/L
13-14 years	60-225 U/L (female) 130-500 U/L (male)
15-17 years	50-140 U/L (female) 60-250 U/L (male)
≥ 18 years	40-120 U/L

*table continued on next page

Table 2-1: Normal reference ranges for variables being investigated in serum and stool…continued

Variable	Normal Reference Range
GGT	
< 1 year	< 100 U/L
1-17 years	< 27 U/L
≥ 18 years	< 50 U/L (female) < 80 U/L (male)
International normalized ratio (INR)	0.9-1.1
Markers of renal function/disease	
Creatinine	
< 2 years	10-40 μmol/L
2-5 years	20-45 μmol/L
6-12 years	20-75 μmol/L
13-14 years	30-85 μmol/L
15-150 years	40-100 μmol/L 50-120 μmol/L (male)

All reference values from Alberta Precision Laboratories (https://www.albertaprecisionlabs.ca/tc/Page13850.aspx), unless otherwise indicated.

Table 3-1. Chronic Kidney Disease Classification[155]

Stage	Description
1	Kidney damage with a normal or increased GFR (> 90 mL/min/1.73m^2)
2	Mild reduction in the GFR (60-80 mL/min/1.73m^2)
3	Moderate reduction in the GFR (30-59 mL/min/1.73m^2)
4	Severe reduction in the GFR (15-29 mL/min/1.73m^2)
5	Kidney failure GFR (< 15 mL/min/1.73m^2)

GFR – glomerular filtration rate

Table 4-1. Variables for statistical analysis

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Outcome variable – Anemia				
Iron deficiency anemia (IDA)	Categorical (present/not present)	Low serum hemoglobin with: • low MCV and, • Ferritin <30 μg/L, and • TSAT < 15% **OR** Low serum hemoglobin with: • Ferritin <30 μg/L, and • sTfR > normal for age, or sTfR-F index >1.5	Proportions	Primary outcome
Anemia of inflammation (AI)	Categorical (present/not present)	Low serum hemoglobin with: • low or normal MCV, and • Ferritin > 100 μg/L, and • TSAT normal or < 20% **OR** Low serum hemoglobin with: • Ferritin > 100 μg/L, and • sTfR $\leq$ normal for age or sTfR-F index $\leq$ 1.5 see Tables 6-B and 7-B for more details	Proportions	
Mixed IDA/AI	Categorical (present/not present)	Low serum hemoglobin with: • low MCV and Ferritin 30-100 μg/L, OR Normal MCV and Ferritin 0-100 μg/L, and • TSAT < 20% OR Low serum hemoglobin with: • Ferritin < 100 μg/L, and • sTfR > normal fo rage, or sTfR-F Index > 1,5 see Tables 6-B and 7-B for more details	Proportions	

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Nutritional Anemia	Categorical (present/not present)	• Low hemoglobin with elevated MCV and: • low vitamin B12, or high MMA, or low folate	Proportions	Primary outcome
No Anemia	Categorical (present/not present)	• normal serum hemoglobin for age	Proportions	
Exposure Variables – Demographic and surgical characteristics				
Age at enrolment	Continuous	Participant age (years) at enrolment	Median and IQR Wilcoxon Rank Sum Test	Younger children at increased risk for anemia due to increased demands.[23]
Sex	Binary/Categorical (male/female)	Participant's biologic sex	Proportions Chi square/Fisher's exact test	Male sex is a risk factor for iron deficiency in young children.[23]
Duration of observation	Continuous	Time of observation in years from date of 1st hemoglobin measurement to end of observation period (ie, study enrolment).	Median and IQR Wilcoxon Rank Sum Test	For use in descriptive statistics and to contextualize chronicity of anemia.
Etiology of intestinal failure (IF)	Categorical (11 categories)	Primary cause of intestinal failure	Proportions Fisher's exact test	Underlying etiology will impact risk for types/causes of anemia (e.g. anastomotic ulcers in SBS, SIBO in motility disorders).
History of STEP	Categorical (yes/no)	History of surgical bowel lengthening procedure	Proportions Chi square	Potential increased risk of ulcers at staple lines.[83, 173]
Presence of Ileocecal valve (ICV)	Categorical (yes/no)	Intact ileocecal valve		Absence of ICV is a risk factor for SIBO.[177]
SBS type I, II or III	Categorical (3 categories)	Intestinal tract anatomy • SBS type I = enterostomy • SBS type II = jejuno-colic anastomosis • SBS type III = jejuno-ileocolic anastomosis	Proportions Fisher's exact test	Resulting GI anatomy is a risk factor for malabsorption and various micronutrient/vitamin deficiencies

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Bowel length - % predicted	Continuous	Remaining bowel length at last surgical procedure or predicted bowel length for gestational age.[178]	Median and IQR Wilcoxon Rank Sum Test	Short bowel is a risk factor for malabsorption.
Exposure Variables – Hematologic investigations[14]				
Hemoglobin	Categorical (low/normal)	Oxygen carrying protein in RBC.	Used for anemia classification purposes	Low hemoglobin is defining criteria for anemia.
MCV	Categorical (low/normal/high)	Average size of RBC.	Used for anemia classification purposes	MCV is used to describe and categorize types of anemia.
Ferritin	Categorical (low/normal/high)	Measure of iron stores in the body. Also an acute phase reactant.	Used for anemia classification purposes	Used in diagnosis of iron deficiency (ferritin <30 μg/L). Levels with increase in the presence of inflammation.
TSAT	Categorical (low/normal)	Measure of iron content in circulating transferrin (iron transport protein). Reflects availability of utilizable iron.	Used for anemia classification purposes	Required for diagnosis of iron deficiency anemia. Low in iron deficiency anemia (<15%). Cut-off for iron deficiency is <20% in presence of inflammation.
Soluble transferrin receptor (sTfR)	Categorical (normal/high)	Measure of number of available transferrin receptors in blood as marker of iron load in cells.	Used for anemia classification purposes	Used to discriminate IDA from AI and mixed IDA/AI, as well as identify iron deficiency before development of anemia in various patient populations.[49–53]
Soluble transferrin receptor/log ferritin index (sTfR-F index)	Categorical (normal/high)	Determined by dividing sTfR by log ferritin value. Marker of iron supply available for erythropoiesis.		

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Exposure Variables - Biomarkers of inflammation				
C-reactive protein (CRP)	Categorical (normal/elevated)	Serum marker frequently elevated in systemic inflammation CRP > 8.0 mg/L = elevated Cut-off of CRP <10mg/L considered normal for evaluation of vitamins/micronutrients.	Used in classification of anemia	Marker of systemic inflammation. Frequently elevated in cases of anemia of inflammation. [76, 179]
Erythrocyte sedimentation rate (ESR)	Categorical (normal/elevated)	Serum marker frequently elevated for age in systemic inflammation Elevated if > 10 mm/hr (ages 0-12yrs) Elevated if > 20 mm/hr (age > 12 yrs female) Elevated if > 15 mm/hr (age >12 yrs male)	Used in classification of anemia	Marker of systemic inflammation. May be elevated in cases anemia of inflammation.[179]
Fecal Calprotectin (FCP)	Categorical (normal/elevated)	Biomarker of inflammation specific to the intestine found in stool. Elevated if > 150 ug/g	Proportions Chi square/Fisher's exact test Univariable odds ratio	Marker of intestinal inflammation.[180] Evidence of elevated levels in various patient population with SIBO, including short bowel syndrome.[74181] May also be elevated in systemic inflammation.[182]
Exposure Variables – IF associated complications				
Small intestine bacterial overgrowth (SIBO)	Categorical (present/not present)	SIBO present based on any of: • Clinical symptoms (see section 3.3) • Positive hydrogen breath test • Positive culture from small bowel aspirate Clinical diagnosis may be supported by histologic evidence of villous blunting, erosions and inflammation.	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	May be a contributor to both iron deficiency anemia and anemia of inflammation. May contribute to intestinal inflammation and GI bleeding.[74,93]

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Liver Disease	Categorical (present/not present) *considered present if present at time or within 6 mos of CBC measurement	• Direct bilirubin > 34 μmol/L for minimum of 2-4 weeks, or • evidence of fibrosis or cirrhosis or portal hypertension on ultrasound +/- doppler or liver elastography, or • Histologic evidence of fibrosis or cirrhosis or inflammation or chronic cholestasis on liver biopsy, not due to another process	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Intestinal failure associated disease may be a cause of anemia of inflammation. [59]
Liver Disease – any history	Categorical (present/not present)	As defined above, but considered present if any history of liver disease, not just at time corresponding to CBC measurement.		Any history included to overcome limitations of disparate timing of investigations
Histologic intestinal inflammation	Categorical (present/not present) *considered present if present at time or within 6 mos of CBC measurement	Considered present if pathologist reports presence of histologic inflammation on intestinal mucosal biopsies obtained during endoscopy.		Histologic inflammation may be a cause of iron deficiency anemia and anemia of inflammation.
Histologic intestinal inflammation – any history	Categorical (present/not present)	As defined above, but considered present if any history of histologic intestinal inflammation, not just at time corresponding to CBC measurement		Any history included to overcome limitations of disparate timing of investigations
GI bleed	Categorical (present/not present) *considered present if bleed presented within 4 weeks of CBC measurement	Evidence of GI bleed if child presents with frank hematemesis or frank/oxidized blood out of G-tube, hematochezia or melena stools.		GI bleed is a known cause of iron deficiency anemia and may be indicative of inflammation in the GI tract as well.

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
GI bleed – any history	Categorical (present/not present)	As defined above, but considered present if any history of GI bleed, not just at time corresponding to CBC measurement	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Any history included to overcome limitations of disparate timing of investigations.
Chronic Kidney Disease (CKD)	Categorical (present/not present)	GFR < 60 mL//min/1.73 m^2 or persistent proteinuria $\geq$ 3 months	Proportions	CKD has been proposed as a contributor to anemia of inflammation in IF.[59]
Exposure Variables – Vitamin and micronutrient levels				
Iron deficiency	Categorical (deficient/not deficient)	Low level of stored iron in cells • Ferritin < 30 μg/L or TSAT < 15% or TSAT < 20% in presence of inflammation*	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	First stage of iron deficiency anemia.[23]
Vitamin D deficiency	Categorical (deficient/not deficient)	Low vitamin D level (25-OH vitamin D) (< 25 nmol/L)		Vitamin D deficiency is associated with both IDA and AI.[107] [108][126]
Vitamin D insufficiency	Categorical (sufficient/insufficient)	Low vitamin D level (25-OH vitamin D) (25-50 nmol/L)		
Vitamin B12 deficiency	Categorical (deficient/not deficient)	Low serum vitamin B12 (< 155 pmol/L) or elevated MMA (> 0.4 μmol/L)		Defining criteria for nutritional anemia.[131]
Folate deficiency	Categorical (deficient/not deficient)	Low serum folate level (< 12.1 nmol/L)		Defining criteria for nutritional anemia.[131]
Copper deficiency	Categorical (deficient/not deficient)	Low serum copper level (11 μmol/L)		Important cofactor in several enzymes and metabolic processes involved in hemoglobin synthesis and iron oxidation. Copper competes with iron and zinc for absorption. [119][120]

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Zinc deficiency	Categorical (deficient/not deficient)	Low serum zinc level (< 8 μmol/L)	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Important cofactor in several enzymes and metabolic processes involving iron metabolism and erythropoiesis. Zinc competes with iron and copper for absorption.[109110111]
Low ceruloplasmin	Categorical (low/normal)	Low serum ceruloplasmin level <0.16 g/L (female); < 0.15 g/L (male)		Low in the presence of severe copper deficiency and may indicate liver disease.
Exposure Variables - Nutrition				
Parenteral Nutrition (PN)	Categorical (present/not present)	Use of PN at time of CBC measurement.	Proportions Univariable/multivariable odds ratio	PN use may contribute to anemia of inflammation and can be a risk factor for
PN duration	Continuous	Time (in months) of use of parenteral nutrition during the study period.	Median and IQR	Longer PN duration previously shown to be associated with risk of micronutrient deficiencies and risk of renal and liver disease.[5759858656]
Exposure Variables – Medications				
Antibiotics for SIBO prophylaxis or treatment	Categorical (present/not present)	Use of oral antibiotics for SIBO therapy or prophylaxis at time of CBC measurement.	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Antibiotics may indicate presence of SIBO. Their use may improve SIBO and risk of anemia or could contribute to worsening dysbiosis and inflammation.

Variable	Variable Type	Variable Definition	Variable Analysis	Variable Justification
Proton pump inhibitor (PPI)	Categorical (present/not present)	Use of PPI at time of CBC measurement.	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	PPI use may contribute to iron deficiency anemia and anemia of inflammation by inhibiting iron absorption and promoting SIBO.
Exposure Variables – Supplements/Therapies				
Iron supplementation	Categorical (present/not present)	Oral, intravenous or PN supplementation of iron	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Iron supplementation (PO/IV) indicative of anemia. Iron-supplemented PN may impact rates of anemia and contribute to inflammation if causing iron overload.
Packed Red Blood Cell (PRBC) Transfusion	Categorical (present/not present)	Transfusion of packed red blood cells	Proportions Chi square/Fisher's exact test	Use is suggestive of GI bleed requiring intervention.
Exposure Variables – Intestinal findings on endoscopy				
Anastomotic ulcer (AU)	Categorical (present/not present)	Endoscopic visualization of ulcers at or around the site of intestinal anastomosis.	Proportions Chi square/Fisher's exact test Univariable/multivariable odds ratio	Anastomotic ulcers may be a source of GI blood loss and contribute to iron deficiency anemia and may also be involved in anemia of inflammation.[82,83]
Endoscopic evidence of staple line ulcers	Categorical (present/not present)	Endoscopic visualization of staple line ulcers.		Staple line ulcers may contribute to anemia in similar methods to anastomotic ulcers.[82,83]

AI=anemia of inflammation; AU=anastomotic ulcer; CKD=chronic kidney disease; CRP=C-reactive protein; ESR=erythrocyte sedimentation rate; ICV=ileocecal valve; IDA=iron deficiency anemia; IF=intestinal failure; IQR=interquartile range; MCV=mean corpuscular volume; MMA= methylmalonic acid; PN=parenteral nutrition; PPI=proton pump inhibitor; PRBC=packed red blood cells; SBS=short bowel syndrome; SIBO=small intestine bacterial overgrowth; STEP=serial transverse enteroplasty; sTfR=soluble transferrin receptor; TSAT= transferrin saturation;