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Effect of Iron Deficiency on Short-Term Response to Treatment in Cats With Chronic Enteropathies

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ABSTRACT

Background: Iron deficiency in humans with chronic inflammatory enteropathies (CIE) is associated with active disease and anemia-related morbidity.

Objectives: To compare iron deficiency prevalence in cats with CIE versus low-grade alimentary lymphoma (LGAL) and secondarily, determine the effect of iron deficiency on short-term clinical response in CIE cats.

Animals: Twenty-eight client-owned cats with primary gastrointestinal disease, including 14 CIE cats and 14 LGAL cats.

Methods: Prospective study. Cats were enrolled when they presented for gastrointestinal endoscopy. Iron panel (serum iron, ferritin, total iron binding capacity), CBC, cobalamin, serum amyloid A, methylmalonic acid, and clinical disease severity were evaluated. Cats were categorized as "normal" or "iron deficient" using calculated transferrin saturation. CIE cats were reevaluated 14, 30, and 90 days after initiation of non-standardized gastrointestinal disease treatment. Clinical response was compared based on iron and anemia status.

Results: Iron deficiency was diagnosed in 7/28 cats (2/14 CIE, 5/14 LGAL cats) at enrollment and developed in four additional CIE cats during follow-up. While 9/28 cats were anemic at enrollment, neither anemia (relative risk [RR], 0.90; 95% confidence interval [CI], 0.13–5.50) nor iron status (RR, 0.4; 95% CI, 0.10–1.5) was associated with treatment response. Transferrin saturation was lower in LGAL cats (22%; 95% CI, 20%–27%) than in CIE cats (30%; 95% CI, 26%–39%).

Conclusions and Clinical Importance: There was high prevalence of iron deficiency over the first 90 days of treatment in CIE cats. Iron deficiency did not affect short-term response to individualized treatment of gastrointestinal disease.

1 | Introduction

Iron deficiency develops due to multiple mechanisms in chronic gastrointestinal (GI) disease, and GI disease can result in both absolute and functional iron deficiency (FID). Absolute iron deficiency (AID) occurs with decreased intestinal uptake or GI loss, whether due to hyporexia or therapeutic diets, decreased absorption, or GI bleeding [1, 2]. Impaired iron absorption in humans with chronic inflammatory enteropathies (CIEs) is due to GI mucosal and systemic inflammation. Serum inflammatory

Abbreviations: %HypoRetic, percentage of hypochromic reticulocytes; AID, absolute iron deficiency; BCS, body condition score; CE, chronic enteropathy; CHR, reticulocyte hemoglobin content; CIE, chronic inflammatory enteropathy; CKD, chronic kidney disease; CR, complete response; DSS, disease severity scores; FID, functional iron deficiency; FS, fecal score; GI, gastrointestinal; Hct, hematocrit; Hgb, hemoglobin; LGAL, low-grade alimentary lymphoma; LP, lamina propria; MCS, muscle condition score; MCV, mean corpuscular volume; MMA, methylmalonic acid; PARR, PCR for antigen receptor rearrangement; RBC, red blood cell; ReticMCV, reticulocyte mean corpuscular volume; SAA, serum amyloid A; TIBC, total iron binding capacity; TSAT, percent transferrin saturation; WSAVA, World Small Animal Veterinary Association.

markers inversely correlate with oral iron absorption [2]. Circulating inflammatory markers correlate with hepcidin concentrations, with increased hepcidin promoting ferroportin internalization, trapping iron within enterocytes. Iron is then lost with enterocyte sloughing, rather than being absorbed from the GI tract [2–4]. In humans with CIE, there is lower serum ferritin and higher inflammatory markers associated with active disease versus remission [2–4]. Inflammation also results in ferritin internalization within reticuloendothelial cells, preventing export into the blood (i.e., FID with adequate total body iron). Assessment of inflammation helps distinguish FID and anemia of inflammation from AID in humans and dogs [5–7]. Serum amyloid A (SAA) is an inflammatory biomarker in cats, and increases are associated with lower total iron binding capacity (TIBC) [8]. Due to difficulty distinguishing FID from AID in humans with CIE, multiple measurements are used, and some individuals have contributions from both [7]. Transferrin saturation (TSAT) < 20% is a marker of iron deficiency, with concurrently decreased ferritin used to define AID [7]. Individuals with normal to elevated ferritin are diagnosed with FID; low-normal ferritin concentrations suggest combined FID and AID due to the potential for increased ferritin concentrations as an acute phase protein, counteracting otherwise decreased concentrations [7].

While the pathogenesis of anemia in individuals with chronic enteropathies (CEs) is multifactorial, both AID and FID contribute to the anemia identified in 80%–90% of humans with CIE [9, 10]. Decreased iron delivery to erythroid precursors results in diminished response to erythropoietin. Inflammation increases red blood cell (RBC) phagocytosis and decreases RBC lifespan, exacerbating anemia. Historically, iron supplementation was only prescribed for human CIE patients with AID. However, the association between iron deficiency and secondary morbidity due to anemia is well recognized in CIE individuals. Therefore, iron supplementation is recommended for all anemic human CIE patients, including those with FID [7, 11–13]. Although the connection between iron deficiency and morbidity in humans with GI lymphoma is not thoroughly described, there is a similar association with CIE progression to GI T-cell lymphoma, as is observed in cats, likely driven by chronic antigenic stimulation [14]. Iron deficiency is also observed with human GI lymphoma [14–16]. Iron homeostasis in feline CE has been minimally explored. In one study, 7 out of 20 cats were diagnosed with FID [14]. Hematocrit (Hct), reticulocyte hemoglobin content (CHR), and TSAT correlated positively with serum iron [17].

Iron deficiency prevalence, FID or otherwise, has not been compared between cats with CIE and low-grade alimentary lymphoma (LGAL) at the time of diagnosis, nor has iron deficiency's effect on short-term outcomes of feline CIE been evaluated. The primary study aim was to compare the prevalence of iron deficiency in cats with CIE versus LGAL at the time of CE diagnosis. A secondary objective, in light of previous serum iron associations with CBC findings [17], was to determine the effect of iron deficiency on short-term response to standard-of-care treatment in CIE cats, including cats with low iron but normal or undefined ferritin. As LGAL is generally considered a progression from feline lymphoplasmacytic CIE, we hypothesized that more cats with LGAL than CIE would have iron deficiency. We also hypothesized that CIE cats with iron deficiency would have higher clinical disease severity scores (DSS) over time versus cats without iron deficiency.

2 | Materials and Methods

Client-owned cats presenting to the Veterinary Health Center at Kansas State University and The Ohio State University Veterinary Medicine Center for chronic GI disease were enrolled prospectively and concurrently with GI endoscopy to obtain a definite diagnosis for their clinical signs between June 2021 and April 2023. The Institutional Animal Care and Use Committees at Kansas State University and The Ohio State University approved all study procedures (Protocols 4439 and 2021A00000015).

2.1 | Study Cohort

Adult cats were enrolled following a standard work-up and diagnosis of primary CE, with exclusion of systemic causes of clinical signs, including at minimum 4 weeks' duration of or recurrent signs of GI disease (vomiting, diarrhea, dysrexia, weight loss), CBC, serum biochemistry profile, and urinalysis. All cats had total thyroxine (tT4) concentrations measured within 3 months and following onset of signs of GI disease, as well as trypsin-like immunoreactivity and abdominal ultrasonography within 1 month of enrollment. Cats with exocrine pancreatic insufficiency, uncontrolled hyperthyroidism, or other documented causes of clinical signs (e.g., IRIS Stage 2 or higher CKD, heart disease requiring medical therapy, infectious disease, or neoplasia exclusive of LGAL) were excluded. Cats with controlled hyperthyroidism based on tT4 with either medical management or after I-131 therapy were allowed. Pancreatic specific lipase was analyzed at clinician discretion, and cats with elevated results were allowed. Fecal flotation, *Giardia* antigen, or feline retroviral testing were performed at clinician discretion. Treatments resulting in exclusion were blood transfusion, steroids, cobalamin, iron, or hematopoietic stimulating agent (e.g., darbepoetin alfa) administration within 1 month of enrollment. Owners completed a questionnaire for standardized clinical DSS based on the feline CE activity index [18], which included diet, supplement, and medication history (Supporting Information 1). Anemia was defined as Hct below the laboratory reference interval (< 35%). Iron deficiency was diagnosed using human guidelines based on low serum iron and TSAT < 20% [7]. In cats where ferritin measurement was available, iron deficiency was further classified as either: (1) FID: ferritin ≥ 82 ng/mL + TSAT < 20% or (2) AID: ferritin < 82 ng/mL (feline reference interval, 82–395 ng/mL) + TSAT < 20% [7].

2.2 | Blood and Histopathology Sampling

At enrollment, whole blood was collected at a volume less than 5% total circulating volume based on an estimated 55 mL/kg. CBC with reticulocyte indices was performed immediately on EDTA anticoagulated whole blood using an in-house analyzer (Advia 2120i Hematology System; Siemens HealthCare GmbH, Munich, Germany). Blood for serum analyses clotted at room temperature and then was centrifuged at 1500*g* for 20 min and separated immediately. Serum for iron and TIBC was stored at 4°C and assays performed within 1 week through the Kansas State University Veterinary Diagnostic Laboratory (KSDVL; Hitachi Cobas c501; Roche Diagnostics, Basel, Switzerland).

Ferritin was measured in a subset of cats using a feline-specific ELISA (commercially available through the KSVDL). Serum for methylmalonic acid (MMA) and SAA concentrations [19] was stored at −80°C and analyzed in bulk at study completion (Texas A&M Gastrointestinal Laboratory, College Station, Texas and University of Miami Acute Phase Protein Laboratory, Miami, Florida, respectively).

All cats underwent upper and lower GI endoscopy to obtain a histopathologic diagnosis. Histopathologic evaluations were performed by board-certified veterinary anatomic pathologists (B.L.P., A.K.F.). Diagnosis was based on World Small Animal Veterinary Association (WSAVA) scoring criteria [20]. WSAVA criteria assign morphologic and inflammatory scores to each GI tract segment. Morphologic components include evaluations of epithelial injury, fibrosis, villus stunting, and intestinal crypt and lacteal abnormalities. Inflammatory components include gastric lymphoid follicular hyperplasia, intraepithelial lymphocytes, and lamina propria infiltration of leukocytes. Scores ranging from 0 (normal) to 3 (marked increase), or in reference to colonic goblet cells, from marked decrease (dec2) to marked increase (inc2) in either direction from normal (0) were assigned. When necessary to clarify the diagnosis, PCR for antigen receptor rearrangement (PARR) through Colorado State University for immunoglobulin and T-cell receptor genes [21] and/or immunohistochemistry for CD3+ lymphocytes were performed on an individual basis. Immunohistochemistry was used to highlight severity (number of intraepithelial lymphocytes) and distribution (single cells, nests [> 5 clustered], or plaques [> 5 epithelial cells occluded by lymphocyte clusters]) of T-cell lymphocytes within the epithelial layer and utilized for diagnosis classification (i.e., CIE or LGAL) when histopathology and PARR were discordant [22]. PARR results, in conjunction with histopathologic and immunohistochemical findings, were used to diagnose cats with LGAL.

2.3 | Treatment and Study Follow-Up

Treatments initiated by clinician discretion at enrollment included one or more diet trials with novel protein or hydrolyzed diets, administration of prednisolone, and administration of cyanocobalamin. Cats with a CIE diagnosis had rechecks performed at 14, 30, and 90 days. Cats with inadequate treatment response based on clinical DSS, fecal scoring (FS), and clinician interpretation at any study recheck were allowed therapeutic adjustments. No cats received iron supplementation during the study. Physical examination, including body weight, body condition score (BCS) [23], muscle condition score (MCS) [24], CBC, iron panel with ferritin when possible, MMA, and SAA, clinical DSS [18], and FS [25] were evaluated at rechecks. As part of DSS, gross endoscopic lesions were noted as present or absent. As repeat endoscopy and histopathologic evaluation were not performed at rechecks, cats received the same component score as their baseline score for this DSS value.

2.4 | Statistical Analysis

Statistical analysis was performed using commercial software (GraphPad Prism Version 10.1.2, GraphPad Software). Data were evaluated for normality using the Shapiro–Wilk test. Data are presented as mean ± standard deviation (SD) and median (range or 95% confidence interval [CI]) for normally distributed and non-normally distributed data, respectively. Study group characteristics (sex, age, weight, BCS, and duration and severity of signs of GI disease) were evaluated using descriptive statistics. Baseline DSS, iron indices, hematologic variables, SAA, and cobalamin variables were compared between cat disease groups, iron groups, and anemic/non-anemic cats using the Mann–Whitney *U* test.

Differences in variables over time were compared in CIE cats using a mixed-model with repeated measures, followed by pairwise testing with corrected p values in statistically significant models. Complete disease response (CR) was defined as a clinical DSS of 0–5 denoting mild disease [26] or ≥ 75% reduction in cats with initial scores ≤ 5. Partial response was defined as a 50%–74% reduction in DSS [27]. The Fisher exact test compared the proportion of cats achieving CR at 90 days between cats with/without iron deficiency.

Spearman's correlation (r_s) was used to compare the duration of signs of GI disease, DSS, and laboratory variables. The strength of correlation was defined as: 0–0.09 = negligible, 0.1–0.39 = weak, 0.4–0.69 = moderate, 0.7–0.89 = strong, 0.9–1.0 = very strong [28]. $p < 0.05$ was considered significant unless otherwise noted for multiple comparison correction.

3 | Results

3.1 | Study Group and Clinical Signs of GI Disease

Twenty-eight client-owned cats, 19 spayed females and 9 castrated males, were enrolled at the time of histopathologic diagnosis of CIE ($n = 14$) or LGAL ($n = 14$) and exclusion of non-GI disease. Two cats had a previous hyperthyroidism diagnosis ($n = 1$, methimazole; $n = 1$, I-131 3 years before); both had a normal tT4 at enrollment. Diagnosis was made by histopathology alone ($n = 5$ CIE, $n = 2$ LGAL), combined histopathology and PARR ($n = 7$ CIE, $n = 6$ LGAL), and combined histopathology, PARR, and immunohistochemistry ($n = 2$ CIE, $n = 6$ LGAL). Breeds included domestic shorthair ($n = 14$), domestic medium hair ($n = 5$), domestic longhair ($n = 2$), Siamese ($n = 2$), Sphynx ($n = 2$), and one each Himalayan, Maine Coon, and Persian. Median age was 10.7 years (range, 1.0–15.3), weight was 4.5 kg (range, 2.1–5.4 kg), and BCS was five out of nine (range, 2–7). Eighty-six percent (24/28) of cats had normal muscling to mild diffuse muscle atrophy, whereas three cats had moderate diffuse muscle atrophy, and one cat had severe, diffuse muscle atrophy. Cat demographics are presented in Supporting Information 2.

Median duration of clinical signs of GI disease was 8 months (range, 1–132). The most common presenting clinical sign was vomiting in 79% ($n = 22$) of cats, followed by weight loss in 68% ($n = 19$), diarrhea in 46% (FS ≥ 3.5; $n = 13$), and variable degrees of hyporexia to anorexia in 36% ($n = 10$). Nine cats (32%) had lost over 10% of body weight since the onset of their clinical signs. Mean presenting clinical DSS was 6.1 out of 19 ± 3.2. WSAVA scores are presented in Supporting Information 3.

3.2 | Iron Status and Cat Outcomes

Complete (iron, ferritin, TIBC) iron panels were performed in 18 of 28 cats (64%); while ferritin analysis was not performed in 10 cats due to discontinuation of the ferritin assay and lack of alternative assays. Cats that did not have ferritin measurements were classified into normal or iron-deficient groups, but not further categorized into AID versus FID groups. Based on TSAT, 21 cats had normal iron status, and 7 cats had iron deficiency. Of the seven cats with iron deficiency, four had serum ferritin concentrations evaluated, allowing classification of FID in three and AID in one CIE cat. Iron deficiency was diagnosed at enrollment in five LGAL cats and two CIE cats (relative risk iron deficiency in CIE cats 0.4; 95% CI, 0.10–1.5; $p = 0.39$). There was no difference in serum iron ($p = 0.06$), ferritin ($p = 0.55$), or TIBC ($p = 0.98$) between LGAL and CIE cats. TSAT was lower in LGAL cats ($p = 0.02$; Table 1; Supporting Information 4). Endoscopic lesions were observed in 11 out of 28 (39%) cats ($n = 2$ iron deficient; $n = 9$ normal iron). Three cats, all in the normal iron group, had superficial, pinpoint gastric ($n = 1$) or duodenal ($n = 2$) erosions; no cat had overt GI ulceration.

Follow-up was available for 13 of 14 CIE cats; while 1 cat in the normal iron group died from GI disease before the 14-day recheck. Of cats with follow-up, nine achieved CR, including five at 14 days, two at 30 days, and two at 90 days; one achieved partial response at 90 days. Three cats were nonresponsive to treatment with < 50% improvement in clinical DSS. Clinical DSS were lower at all rechecks compared to baseline (model $p < 0.001$; $F[1.394, 14.87] = 15.18$; Figure 1).

There was no difference in baseline clinical DSS between responders (CR or partial response; median, 9; 95% CI, 4.5–10.7; range, 2–14) and nonresponders (median, 3; 95% CI, 0.8–7.6; range, 2–9; $p = 0.12$). There was no difference in the proportion of cats that achieved CR between those with and without iron deficiency (RR, 1.3; 95% CI, 0.6–7.3; $p = 1.0$). There was no difference in baseline iron ($p = 0.87$), ferritin ($p = 0.73$), or TSAT ($p = 0.72$) between CIE cats that achieved CR and those achieving partial or no response. These values did not achieve significance with or without including the cat that died before recheck, so baseline data from that cat was included (Figure 2).

Six total CIE cats had iron deficiency across the study period. While iron deficiency resolved at Day 14 in both CIE cats that were initially iron deficient, two additional cats developed iron deficiency at that recheck, with one of those cats demonstrating CR to treatment. Six cats were iron deficient at 30 days, including both cats that were iron deficient at enrollment and the additional two cats that were iron deficient at Day 14. Two cats were iron deficient at the 90-day recheck, including one of the cats that had newly developed iron deficiency at 30 days and one cat that was iron deficient on Days 14 and 30. Both iron-deficient cats at the 90-day recheck were considered treatment responders; four out of six iron-deficient cats at the 30-day recheck were considered treatment responders.

3.3 | Hematologic Variables

Non-regenerative anemia was present in 32% (9/28) of cats at enrollment, including four CIE cats and six LGAL cats. Enrollment Hct was not different between CIE (median, 36%; 95% CI, 31–41) and LGAL cats (33%; 95% CI, 29–37; $p = 0.23$). Hct was also not different at enrollment between cats with (38%; 95% CI, 32–43) or without (34%; 95% CI, 30–38) iron deficiency ($p = 0.23$). There was no difference in Hct over time in CIE cats ($F[1.302, 13.88] = 0.81$; $p = 0.42$). Presenting Hct was not different in CIE cats with CR (42%; 95% CI, 33–45) versus partial or no response (33%; 95% CI, 18–43; $p = 0.12$; RR, 0.90; 95% CI, 0.13–5.50; $p = 1.0$). Hematologic variables were not associated

TABLE 1 | Iron indices in cats with CIE ($n = 14$) and LGAL ($n = 14$). Ferritin values are included for nine each of the same CIE and LGAL cats, respectively.

Iron variable (feline reference interval)	Median		95% Confidence interval		
	CIE	LGAL	CIE	LGAL	*p*
Iron (65–162 μg/dL)	76	63	68–101	53–73	0.06
Ferritin (82–395 ng/mL)	144	230	52–479	142–335	0.55
TIBC (250–470 μg/dL)	267	262	236–300	242–298	0.98
TSAT (%) *none*	30	22	26–39	20–27	0.02

Abbreviations: CIE, chronic inflammatory enteropathies; LGAL, low-grade alimentary lymphoma; TIBC, total iron binding capacity; TSAT, transferrin saturation.

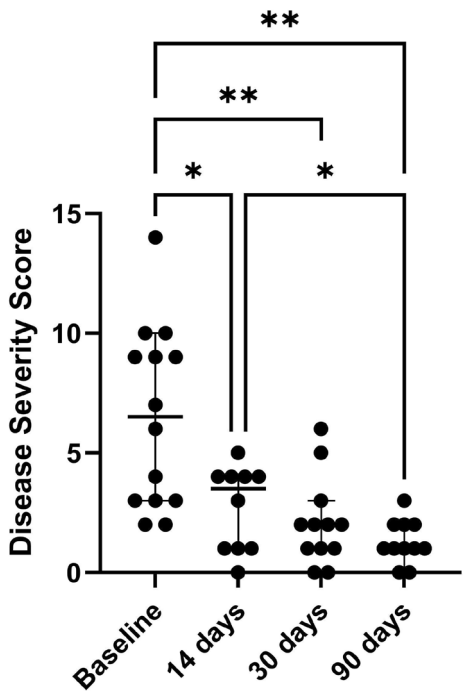
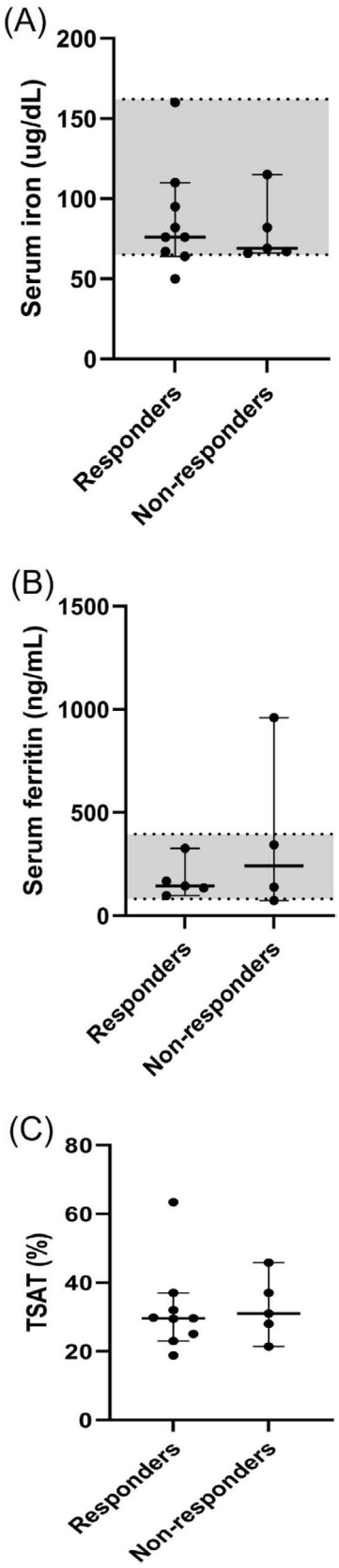


FIGURE 1 | A combined scatter and whisker plot showing serial clinical disease severity scores in cats with CIE as individual data points (circles) and median and 95% confidence interval (bars). Scoring scale ranges from 0 (no disease) to 19 (severe disease). * denotes $p < 0.05$; ** denotes $p < 0.01$. CIE, chronic inflammatory enteropathies.

with any iron indices (Bonferroni adjusted p value for significance < 0.002; Table 2). Neither median TSAT nor serum iron concentrations were different between anemic (TSAT: 24%; 95% CI, 21–27; iron: 63 μg/dL; 95% CI, 51–83) and non-anemic cats (TSAT: 30%; 95% CI, 23–35; $p = 0.38$; iron: 75 μg/dL; 95% CI, 61–93; $p = 0.79$).



3.4 | Iron Indices and Inflammation

Median SAA concentrations were not different between cats with (2.6 mg/L; range, 1.2–7.5) or without iron deficiency (1.8 mg/dL; range, 0.7–23.0; $p = 0.56$) or between LGAL (2.7 mg/dL [0.7–21.6]) and CIE cats (1.6 mg/dL [1.2–23.0; $p = 0.68$]). Based on a cut-off of > 20 mg/L, two cats had elevated SAA, including one CIE cat and one LGAL cat. No cats had an SAA concentration > 10 but < 20 mg/L. Additionally, SAA concentrations were not associated with DSS ($r_s = -0.30$; 95% CI, −0.41 to 0.36; $p = 0.88$) or iron indices. (Table 3) SAA concentrations did not differ among study time points in CIE cats ($F[1.33, 13.31] = 1.14$; $p = 0.33$) and were not different in anemic (1.7 mg/dL; 95% CI, −1.4 to 9.6) and non-anemic cats (2.2 mg/dL; 95% CI, 1.9–6.6; $p = 0.27$).

Intestinal inflammatory WSAVA scores were not different in any anatomic GI region between cats with or without iron deficiency (Table 4).

3.5 | Cobalamin and MMA

At enrollment, 11% ($n = 3$) to 25% ($n = 7$) of cats were hypocobalaminemic based on elevated serum MMA or decreased cobalamin concentrations, respectively. Neither MMA nor cobalamin concentrations were different between CIE and LGAL cats (MMA $p = 0.93$; cobalamin $p = 0.47$). There was no difference in serum MMA or cobalamin concentrations between iron deficient (cobalamin: 1000 ng/L; 95% CI, 532–1109; MMA: 243 nmol/L; 95% CI, 170–320) and non-iron deficient cats (cobalamin: 472 ng/L; 95% CI, 406–747; $p = 0.10$; MMA: 308 nmol/L; 95% CI, 213–966; $p = 0.40$). All three cats with elevated MMA concentrations had normal iron status.

4 | Discussion

This study augments existing evidence for iron dysregulation as a comorbidity in cats with chronic GI disease. Sixty-four percent of CIE cats had a short-term clinical response to disease treatment, which was not affected by the presence of iron deficiency. Cats with LGAL had significantly lower TSAT at presentation, warranting further investigation in this cohort of cats.

Overall, 25% of cats in this study were diagnosed with iron deficiency at initial evaluation, which is lower than in cats with undefined CE [17]. The reason for the difference between studies is

FIGURE 2 | A combined scatter and whisker plot showing baseline serum iron ($n = 14$; A), ferritin ($n = 9$; B), and TSAT ($n = 14$; C) in cats with CIE as individual data points (circles) and median and 95% confidence interval (bars). Shaded regions denote laboratory specific feline reference intervals, where applicable. Responders denote cats with complete treatment response based on clinical disease severity scores; non-responders denote cats with either partial or no response. CIE, chronic inflammatory enteropathies; TSAT, transferrin saturation.

TABLE 2 | Spearman's correlations (r_s) between hematologic variables and iron indices in 28 cats with CIE or LGAL.

Variables	r_s	95% CI	p
Hct and iron	−0.01	−0.39 to 0.38	0.97
Hct and ferritin	−0.16	−0.59 to 0.35	0.53
Hct and TSAT	0.07	−0.33 to 0.44	0.72
Hct and TIBC	0.08	−0.32 to 0.45	0.70
Hgb and iron	−0.03	−0.41 to 0.36	0.88
Hgb and ferritin	−0.08	−0.54 to 0.41	0.75
Hgb and TSAT	0.04	−0.35 to 0.42	0.82
Hgb and TIBC	0.08	−0.32 to 0.45	0.70
MCV and iron	−0.19	−0.54 to 0.21	0.33
MCV and ferritin	−0.17	−0.60 to 0.34	0.50
MCV and TSAT	−0.16	−0.51 to 0.24	0.43
MCV and TIBC	−0.15	−0.50 to 0.25	0.45
ReticMCV and iron	0.06	−0.37 to 0.46	0.79
ReticMCV and ferritin	−0.24	−0.69 to 0.35	0.42
ReticMCV and TSAT	−0.15	−0.53 to 0.28	0.49
ReticMCV and TIBC	0.23	−0.20 to 0.59	0.69
CHR and iron	−0.02	−0.40 to 0.36	0.91
CHR and ferritin	−0.40	−0.51 to 0.45	0.87
CHR and TSAT	−0.05	−0.43 to 0.34	0.80
CHR and TIBC	0.05	−0.34 to 0.42	0.81
%HypoRetic and iron	0.03	−0.43 to 0.47	0.92
%HypoRetic and ferritin	0.04	−0.59 to 0.64	0.92
%HypoRetic and TSAT	−0.05	−0.49 to 0.41	0.83
%HypoRetic and TIBC	0.09	−0.38 to 0.53	0.69

Abbreviations: %HypoRetic, percentage of hypochromic reticulocytes; CHR, reticulocyte hemoglobin content; CIE, chronic inflammatory enteropathies; Hct, hematocrit; Hgb, hemoglobin; LGAL, low-grade alimentary lymphoma; MCV, mean corpuscular volume; ReticMCV, reticulocyte mean corpuscular volume; TIBC, total iron binding capacity; TSAT, percent transferrin saturation.

TABLE 3 | Spearman's correlations (r_s) between SAA and iron indices in 28 cats with CIE or LGAL.

Variables	r_s	95% CI	p
SAA and iron	−0.31	−0.62 to 0.08	0.11
SAA and ferritin	−0.06	−0.52 to 0.44	0.83
SAA and TSAT	−0.06	−0.43 to 0.33	0.77
SAA and TIBC	−0.35	−0.65 to 0.04	0.07

Abbreviations: CIE, chronic inflammatory enteropathies; LGAL, low-grade alimentary lymphoma; SAA, serum amyloid A; TIBC, total iron binding capacity; TSAT, transferrin saturation.

unclear but could be due to criteria for iron deficiency diagnosis. We classified iron status based on TSAT, consistent with human literature [7, 29, 30]; the previous study defined iron deficiency based on decreased serum iron [17]. If the former criteria were applied to the current study group, 32% would have been iron deficient at enrollment. In some human studies, population-specific reference intervals were used to define iron status versus universal TSAT cut-offs. While we used the TSAT cut-off in this study, further research is required to support one approach versus another (i.e., calculated TSAT versus iron panel variable reference intervals) in cats to most accurately define iron deficiency [31–33]. Alternatively, study group differences could contribute to fewer cats with iron deficiency. The former study did not distinguish between causes of CE [17]; in contrast, this study used GI biopsies to obtain a definite diagnosis. Interestingly, calculated TSAT was lower in cats with LGAL versus CIE cats. While the overall proportion of iron-deficient cats was not different between groups, this could relate to the low number of iron-deficient cats in each group, five LGAL and two CIE, respectively. A larger study group could discover an overt difference between these cohorts. If the previous study had a higher proportion of LGAL cats, that could contribute to the higher prevalence of iron deficiency previously reported. Iron deficiency in humans with uncontrolled CIE is variable, ranging from approximately 35% to almost 80% and an overall estimation of 45% [34–37], making this one of the most common micronutrient deficiencies in humans with CIE [38]. Aside from the above-mentioned study [17] and a case report detailing two dogs with iron deficiency anemia secondary to CIE [39], iron deficiency in association with CE is poorly described in companion animals, making it difficult to compare prevalences between feline and human CIE. Differences in disease pathogenesis likely contribute to the lower prevalence reported in this study compared to reports in human literature. Mucosal hemorrhage and overt ulceration are major characteristics of human CIE, which contribute to anemia and iron deficiency [35]. Substantial GI hemorrhage has historically been considered uncommon in CIE cats, which could contribute to a decreased risk of iron deficiency compared to humans. However, CIE is the definite diagnosis in 54% of cats with an endoscopic diagnosis of GI ulceration, suggesting that a subset of cats might be at greater iron deficiency risk [40]. It is possible that differences in GI iron absorption occur in human and feline CIE. While decreased oral iron absorption is noted in humans with active CIE [2], to the authors' knowledge this has not been evaluated in CE cats. Although it is possible that differences in GI anatomic localization could affect differences in the development of iron deficiency among species, the present study showed no difference in WSAVA scores in various GI tract regions between iron-deficient and non-iron-deficient cats, consistent with human literature [3].

Assessment of iron deficiency in individuals with CE is challenging due to the multiple contributing factors to both AID and FID. While serum iron, ferritin, TIBC, and calculated TSAT allow a straightforward assessment in healthy individuals, systemic inflammation confounds interpretation in those with CE. Due to ferritin's role as a positive acute phase protein, a higher ferritin cut-off is used to categorize iron deficiency in individuals with active disease in some human literature [29, 41]. Although we classified iron deficiency based on calculated TSAT, which does not include ferritin, misclassification of AID as FID can occur if standard ferritin references are used. Recent human literature suggests that serum transferrin receptor (TfR) concentrations more accurately distinguish AID from FID [29], as TfR concentrations are not effected by inflammation. Further,

TABLE 4 | Intestinal inflammation scores (WSAVA) in cats with CIE ($n = 14$) or LGAL ($n = 14$) grouped based on the presence or absence of iron deficiency.

	Median inflammatory WSAVA score		Range		
Gastrointestinal segment	**Normal iron**	**Iron deficient**	**Normal iron**	**Iron deficient**	***p***
Stomach	3.0	3.0	1.0–7.0	1.0–5.5	0.50
Duodenum	5.25	5.0	2.0–7.5	0.0–6.0	0.80
Ileum	6.0	4.0	2.0–8.0	2.0–6.0	0.13
Colon	4.5	3.5	1.0–8.0	3.0–6.5	1.0

Abbreviations: CIE, chronic inflammatory enteropathies; LGAL, low-grade alimentary lymphoma; WSAVA, World Small Animal Veterinary Association.

hepcidin mediates inflammatory-driven FID anemia in humans with CIE [42]. Unfortunately, neither TfR nor hepcidin are readily available biomarkers for analysis in cats. Systemic inflammatory markers can help differentiate between FID and AID but are variably reliable for this purpose in humans with active CE [2, 35, 42]. Species' differences also suggest that biomarkers in humans do not consistently translate to cats [43]. SAA is an acute phase protein in cats and is increased at initial diagnosis in cats with LGAL compared to cats with high-grade GI lymphoma [44–47]. Interestingly, our study showed no difference in SAA between CIE and LGAL cats. As LGAL could be a gradual progression of feline CIE [47, 48], it is possible that this explains the lack of SAA difference between disease groups. Additionally, only two cats had SAA concentrations above what is generally accepted as normal, so it is possible that neither feline LGAL nor CIE is associated with acute systemic inflammation. While the above-mentioned study demonstrated a much higher percentage of cats with LGAL and elevated SAA, a lower concentration threshold was used in that study [44]. Given the lack of association between SAA, clinical DSS, or changes over time in CIE cats, it is possible that SAA is not a good inflammatory marker in these diseases. It is also possible that the low number of cats with elevated SAA in this study supports the low number of cats with iron deficiency. As other inflammatory markers are increased in the feces of CE cats [49], further investigation into the association between different acute and chronic inflammatory biomarkers and iron deficiency in CE cats is required [49].

Treatment response was not related to iron status in CIE cats. In cats where further classification of iron deficiency was possible, only one out of four cats had AID, which could contribute to this observation. However, in humans, both AID and FID are associated with uncontrolled CE versus remission [2, 35, 50]. Treatment response in our study was based on clinical DSS. Different measures of active disease versus remission are utilized in human literature. In some studies utilizing clinical disease scores, systemic inflammation and anemia are two factors commonly associated with iron status, but neither has been definitively associated with severity score indices [35, 51]. Therefore, it is possible that evaluating the effect of iron deficiency on treatment response based on clinical DSS is not the best method in cats. Adding further support to this hypothesis, while DSS improved at each recheck in CIE cats, four additional cats developed iron deficiency during the 90-day study period. Further investigation is required to determine the best marker for disease response or active/inactive disease in relation to iron status in cats. In a human study categorizing CIE as active or inactive based on serum cytokines, an inverse correlation between systemic inflammatory markers and intestinal iron absorption was observed [2]. SAA was used as a marker of systemic inflammation in this study, as increased SAA concentrations were previously associated with inflammation-associated decreases in TIBC in cats with CKD [8]. However, we found that SAA was not associated with clinical DSS or iron indices. This suggests that the pathogenesis of iron deficiency in CIE cats could differ from humans or cats with CKD, or it could indicate that SAA is an insensitive marker of disease in this disease cohort.

Although it did not affect short-term outcome, it is unclear why additional CIE cats became iron-deficient during the study period. Sampling at 2-week intervals in the initial study period is a possible contributor, as iatrogenic iron deficiency can occur in healthy cats with sampling frequency and venipuncture volumes within standard guidelines [52]. It is possible that CE cats have lower regenerative capacity than healthy cats and are at risk to become iron-deficient with frequent venipuncture. It would be ideal to distinguish AID versus FID, but this was not possible due to lack of ferritin assessment in many cats. The effect of administered treatments on iron variables also cannot be excluded. Corticosteroids, which were utilized to treat some cats in this study, increase serum iron and TSAT in dogs [53, 54]. However, if the same hematologic changes occur in cats, we would expect this to confound recognition of iron deficiency. Alternatively, glucocorticoids increase risk for gastric erosions without overt clinical disease in dogs, so the potential for subclinical GI hemorrhage contributing to iron deficiency cannot be excluded [55, 56]. This has not been thoroughly explored in cats. Only CIE cats were serially monitored in this study. As additional CIE cats became iron deficient and a higher number of LGAL cats were iron deficient at enrollment, studies evaluating serial progression of iron indices in LGAL cats and effect on outcome are warranted.

Anemia is the most important consequence of iron deficiency in humans and directly contributes to CIE morbidity [12, 13]. While the proportion of cats with anemia in our study (32%) is lower than the 40%–80% reported in human CIE literature [13, 35, 51], it is similar to the 35% anemia prevalence reported in hospitalized cats [57]. Unlike in humans with uncontrolled CIE, where anemia is related to low iron, transferrin, and ferritin [35], no association between iron indices and hematologic variables was identified in our study group. This differs from other studies in cats and dogs, where variable associations

between reticulocyte and iron indices have been described [17, 52, 58–62]. However, the lack of difference in iron status between anemic and non-anemic cats was also observed in the aforementioned study of hospitalized cats [57]. No reticulocyte indices differentiate FID from AID; therefore, this study further supports that direct iron assessment by measuring serum iron and ideally, ferritin concentrations is necessary to determine iron status in cats [60, 61, 63]. In our CIE cats, anemia was not associated with treatment response. Iron deficiency (77%) in some human study cohorts is more common than iron deficiency anemia (44%), so it is possible that further consequences of anemia could be seen in a study cohort with higher prevalence of iron deficiency [51].

Although there was no difference between MMA concentrations in cats with low versus normal iron status, all three cats with an elevated MMA had normal iron status. This would seem consistent with a previous finding of lower TIBC in CE cats with increased MMA [17]; though, the same relationship with TIBC was not identified in this study. This relationship appears to contrast with descriptions in some human diseases where iron deficiency and subsequent mitochondrial dysfunction result in cobalamin deficiency and progressive iron deficiency [64, 65]. Findings suggest that further investigation into the relationship between iron deficiency and cobalamin deficiency in CE cats is needed. It is possible that elevated MMA was unrelated to iron status and a larger study sample size would demonstrate an even distribution between groups. Elevated MMA could alternatively reflect disease severity and localization, as two of these three cats had relatively high combined ileal WSAVA scores of 13, with the third cat having a score of 7.

This study had several limitations. There were a small number of cats with either CIE or LGAL; larger studies are needed to clarify the presence or lack of differences between disease subsets. The initial sample size calculation was based on differences in response (CR vs. partial or no response) in CIE cats with or without iron deficiency. The estimate was based on a difference of 80% CR and 20% CR in cats with and without iron deficiency, respectively, based on the presence of iron deficiency in active versus inactive human CIE [66]. That estimate would require 10 CIE cats in each group (with/without iron deficiency). As the percentage of enrolled cats with iron deficiency was lower than anticipated, this study was likely underpowered to identify differences in treatment response. Due to ferritin assay unavailability during the study, ferritin was only measured in 10 out of 28 cats. While this ultimately did not change the overall assessment of iron status, it limited the ability to distinguish FID from AID in all cats and the etiology of progressive iron deficiency development in some CIE cats. Finally, treatment in CIE cats was not standardized, and the effect on serial iron, hematologic, and inflammatory markers cannot be determined.

In conclusion, while iron deficiency is present in some CE cats, it did not affect short-term response to non-standardized GI disease treatment for CIE.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Kansas State University (protocol number 4439) and The Ohio State University (protocol number 2021A00000015) Institutional Animal Care and Use Committees. Authors declare human ethics approval was not needed.

Conflicts of Interest

Brandon L. Plattner is employed by the Kansas State Veterinary Diagnostic Laboratory, which offers measurement of iron indices on a fee-for-service basis. Alexandra K. Ford was employed by the KSVDL during the study period. The other authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.