Adipokine Resistin Levels at Time of Pediatric Crohn Disease Diagnosis Predict Escalation to Biologic Therapy

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Background: Hypertrophy of visceral adipose tissue (VAT) is a hallmark of Crohn disease (CD). The VAT produces a wide range of adipokines, biologically active factors that contribute to metabolic disorders in addition to CD pathogenesis. The study aim was to concomitantly evaluate serum adipokine profiles and VAT volumes as predictors of disease outcomes and treatment course in newly diagnosed pediatric patients with CD.

Methods: Pediatric patients ages 6 to 20 years were enrolled, and their clinical data and anthropometric measurements were obtained. Adipokine levels were measured at 0, 6, and 12 months after CD diagnosis and baseline in control patients (CP). The VAT volumes were measured by magnetic resonance imaging or computed tomography imaging within 3 months of diagnosis.

Results: One hundred four patients undergoing colonoscopy were prospectively enrolled: 36 diagnosed with CD and 68 CP. The serum adipokine resistin and plasminogen activator inhibitor (PAI)-1 levels were significantly higher in patients with CD at diagnosis than in CP. The VAT volume was similar between CD and CP. Baseline resistin levels at the time of diagnosis in patients with CD who were escalated to biologics was significantly higher than in those not treated using biologic therapy by 12 months (29.8 ng/mL vs 13.8 ng/mL; $P = 0.004$). A resistin level of ≥ 29.8 ng/mL at the time of diagnosis predicted escalation to biologic therapy in the first year after diagnosis with a specificity of 95% (sensitivity = 53%; area under the curve = 0.82; $P = 0.015$ for model with log-scale). There was a significantly greater reduction in resistin ($P = 0.002$) and PAI-1 ($P = 0.010$) at the 12-month follow-up in patients on biologics compared with patients who were not treated using biologics.

Conclusions: Serum resistin levels at diagnosis of pediatric CD predict the escalation to biologic therapy at 12 months, independent of VAT volumes. Resistin and PAI-1 levels significantly improved in patients with CD after treatment using biologics compared with those not on biologics. These results suggest the utility of resistin as a predictive biomarker in pediatric CD.

Key Words: Crohn disease, adipokines, resistin, visceral adipose tissue

INTRODUCTION

Although conventional evaluation of disease activity in Crohn disease (CD) via serum markers, abdominal imaging, and endoscopy is an essential mainstay of disease assessment, there is a need for markers that have better prognostic value for predicting disease outcomes.^{1, 2} Patients diagnosed with CD in childhood endure a greater disease burden than adults with increased risk of recurrent surgery, high rates of disease extension, decreased height potential and bone density, delay in puberty, and increased risk of both cancer and mortality.³⁻⁵ Therefore, it is important to identify additional parameters to risk-stratify pediatric patients with CD at diagnosis and to help in the selection of the most effective first-line therapy, thereby hopefully reducing the risk of future complications.

Hypertrophy of visceral adipose tissue (VAT) accompanying intestinal inflammation has long been recognized as a feature of CD,⁶ and this mesenteric fat also seems to have a negative impact on the disease.⁷ We and others have previously reported that pediatric patients with CD, with or without obesity, have a higher relative proportion of VAT, which is associated with adverse outcomes such as decreased time to surgery and higher rates of hospitalizations.^{8, 9} Adipose tissue has a wide range of biological effects at the metabolic, biochemical, immune, inflammatory, and microbial levels.^{7, 10}

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These effects are mediated through various mechanisms, including the secretion of biologically active molecules known as adipokines.^{11, 12} The main cell types in adipose tissue, including macrophages, pre-adipocytes, and adipocytes, contribute to intestinal inflammatory responses, some of which are mediated by the action of secreted adipokines, which have all been implicated in the role of CD-related intestinal inflammation.^{13, 14} In particular, the adipokines resistin, leptin, adiponectin, and plasminogen activator inhibitor-1 (PAI-1) influence various inflammatory pathways through the activation of Th1 and Th17 cells along with vascular dysfunction.¹⁴ Although VAT volumes and adipokines have been studied independently in CD,¹¹ no studies have attempted to identify a relationship between VAT volumes and adipokine levels. Therefore, the aim of this study was to evaluate serum adipokine profiles and VAT volumes in newly diagnosed patients with pediatric CD at baseline and after treatment in the first year after diagnosis.

PATIENTS AND METHODS

Study Groups

Children aged 6 to 20 years scheduled for colonoscopy for evaluation of gastrointestinal symptoms at Cleveland Clinic Children's Hospital were screened for possible CD (Fig. 1). Patients were excluded if they had a known diagnosis of inflammatory bowel disease (IBD), previous abdominal surgery, diabetes or glucose intolerance, other autoimmune or chronic

inflammatory diseases, or neoplastic diseases. Patients younger than 6 years at diagnosis were excluded to reduce the bias that patients with very-early-onset IBD that may have a monogenic defect with a unique cytokine profile may potentially confound identification of significant results.¹⁵ Based on colonoscopy and histology findings, patients were classified as either control patients (CP) or as patients with CD. The CP were classified based on a normal colonoscopy without chronic inflammation on biopsy. The diagnosis of CD was based on clinical, endoscopic, and histologic criteria.¹⁶ Patients with a disease pattern consistent with ulcerative colitis or indeterminate colitis were excluded from further analysis. Clinical data, anthropometric measurements, and a blood sample before or on the day of colonoscopy were obtained from all enrolled patients.

Disease activity was recorded at diagnosis using the short Pediatric CD Activity Index (sh-PCDAI), a validated index, and categorized according to the Paris classification for CD.^{17, 18} Patients diagnosed with CD were followed at 6 and 12 months after diagnosis for reassessment of clinical data, anthropometric measurements, disease activity, disease outcome, routine laboratory tests, and an additional blood sample. The study was approved by the Cleveland Clinic Institutional Review Board.

Anthropometrics

Anthropometric measurements (weight, body mass index [BMI], and waist circumference) were obtained at the time of enrollment. The BMI percentiles and *z* scores were calculated and adjusted for sex and age from the 2000 Centers for Disease Control and Prevention growth charts.^{19, 20} Waist circumference for sex and age percentile were extrapolated from measures as described by Kuhle et al.²¹ Subsequent anthropometric measurements were obtained for patients diagnosed with CD at the 6- and 12-month follow-ups. Growth failure was defined as: height percentile decreased by 2 isobars *or* height percentile < third percentile for age *or* height velocity < third percentile for age.²²

Adipokine Evaluation

Peripheral blood samples were collected at the time of enrollment (baseline) in all patients and again at the time of follow-up at 6 and 12 months from patients with CD. All samples were collected in a serum separator tube followed by centrifugation (15 minutes at 1000g) for measurement of adipokines and proinflammatory cytokines. Resistin, PAI-1, leptin, adiponectin, hepatocyte growth factor (HGF), tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 concentrations along with respective standards were measured utilizing the commercially available enzyme-linked immunosorbent based Magnetic Luminex Assay (R&D, Minneapolis, MN) on a Luminex 200 Instrument System (Thermo Fisher Scientific, Waltham, MA) per protocol.

CD, Crohn's disease; CS Control Subject; MRE, magnetic resonance enterography; CTE, computed tomography enterography.

FIGURE 1. Screening and study design.

Imaging Analysis

Magnetic resonance imaging (MR enterography/MRE) or computed tomography (CT enterography/CTE) was obtained at the discretion of the primary physician. The VAT volume was measured in every patient using predetermined landmarks from the dome of the diaphragm to the pubic symphysis.

We used MRE Half-Fourier-Acquired Single-shot Turbo (HASTE) Spectral Attenuated Inversion Recovery and HASTE sequences of the intestinal region to segment VAT from the total fat including subcutaneous adipose tissue (SAT) regions through an automated process adapted from Kullberg et al.²³ This process was validated against manual annotations from 2 expert radiologists with excellent accuracy (dice coefficient = 0.86 ± 0.05).

The CT scan images were analyzed using the body fat analysis function from commercial software (Aquarius iNtuition version 4.4.6, TeraRecon Inc., San Francisco, CA) to measure VAT and SAT volumes. The software traces the abdominal wall musculature, separating the visceral from the subcutaneous compartment, and automatically creates the necessary demarcation to measure adipose tissue in the different compartments. Any demarcation errors were manually corrected by a single CT technologist. Aquarius iNtuition displays the outer circumference, the volumes of SAT and VAT, and the volume ratio of VAT to total abdominal fat [VAT/(VAT + SAT)] and VAT:SAT. The range of fat was measured from -50 to -150 Hounsfield units. Aquarius iNtuition has been validated for the measurement of visceral abdominal fat in pediatric patients in a previous study by Blitman et al.²⁴

Statistical Analysis

Data were reported using medians and ranges for continuous variables and counts and percentages for categorical variables. Study groups were compared on demographic and clinical characteristics using Wilcoxon rank sum tests for continuous and ordinal characteristics and χ^2 or Fisher exact tests for categorical characteristics. Correlations between variables were assessed using Spearman rank correlation coefficients. Study groups were compared on log-transformed MRI parameters adjusting for BMI-for-age z scores using logistic regression models. An area under the receiver operator characteristic curve analysis (AUC) was used to assess the potential of resistin, sh-PCDAI, and C-reactive protein (CRP) to predict biologic therapy responses at 12 months. Sample sizes for individual variables reflect missing data. All analyses were performed on a complete-case basis. All tests were 2-tailed and performed at a significance level of 0.05, and SAS 9.4 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Baseline Patient Characteristics

One hundred ten patients were assessed for eligibility at time of colonoscopy during the enrollment period (November

2013–February 2015), including 68 CP and 36 pediatric patients with CD (Fig. 1). In addition, all patients had a concomitant upper endoscopy. Three patients diagnosed with ulcerative colitis and 3 patients with indeterminate colitis were excluded. Thirty-four (94%) patients with CD completed follow-up to 12 months, and 23 (68%) had serum obtained at 12 months. There was no difference in age or sex between the CP and the patients with CD (Table 1). Growth failure was present at diagnosis in 15 (42%) patients with CD compared with 3 (4%) CP ($P < 0.001$). Baseline laboratory values were significantly different in patients with CD compared with CP, including higher CRP (0.8 mg/dL vs 0.1 mg/dL, $P < 0.001$) and erythrocyte sedimentation rate (10.5 mm/h vs 6.0 mm/h; $P < 0.001$) and lower hemoglobin (11.9 g/dL vs 13.5 g/dL; $P = 0.002$) and albumin (3.9 g/dL vs 4.6 g/dL; $P < 0.001$) (Table 1, Supplementary Table 1).

The CD location was terminal ileum $\pm$ limited cecal disease in 25% of patients with CD, colonic in 19% of patients, and ileocolonic in 56% of patients. Fifty percent of patients with CD also had upper tract involvement. Disease phenotype at diagnosis was inflammatory in 94% of patients, with 2 having penetrating disease and 3 with perianal disease. Baseline sh-PCDAI in patients with CD who were followed to 12 months was classified as mild in 53%, moderate in 41%, and severe in 6% (Supplementary Table 2).

Baseline Adipokines and VAT Volume

Baseline adipokine and cytokine levels and VAT and SAT volumes for CS and CD are shown in Table 1. The adipokines resistin and PAI-1 and the cytokines HGF, IL-1 β , and IL-6 were significantly higher in patients with CD than in CP. There were no differences in adiponectin, leptin, and TNF- α levels between patients with CD and CP.

Patients with colonic and ileocolonic disease had significantly higher levels of baseline resistin compared with patients with terminal ileum $\pm$ limited cecal disease (23.1 ng/mL and 23.8 ng/mL vs 12.6 ng/mL; $P < 0.001$). Patients with ileocolonic disease also had higher levels of CRP at diagnosis (Supplementary Table 3).

Imaging was obtained in 25 patients with CD and 17 CP (Fig. 1). The VAT volume, SAT volume, VAT/VAT+SAT and VAT:SAT ratio were similar in CP and patients with CD when adjusted for BMI-for-age z score (Table 1, Supplementary Table 1). In addition, there was no difference in the VAT volume between imaging modalities (MRE, $n = 31$; 478.2 cm³ [95% confidence interval (CI), 106.6–2366.6] vs CTE, $n = 12$; 281.0 cm³ [95% CI, 136.0–683.0]; $P = 0.073$) in all patients. The VAT volume in patients with CD was higher in those who had MRE ($n = 20$; 548.2 cm³) than in those who had CTE ($n = 5$; 199.0 cm³; $P = 0.024$) but without a difference in the VAT:SAT ratio (MRE = 0.5 vs CTE = 0.3; $P = 0.07$).

Baseline Adipokines and VAT Volumes and Subsequent Escalation to Biologic Therapy

By 12 months, 44.1% (15/34) of patients with CD had required biologic therapy as determined by their treating

TABLE 1. Baseline Characteristics of CP vs Pediatric Patients With CD

Factor	CP (n = 68)		Patients With CD (n = 36)		P
	n (%)	Median (minimum-maximum)	n	Median (minimum-maximum)	
Demographics					
Age at enrollment (y)	68	14.5 (6.0-20.0)	36	13.0 (7.0-18.0)	0.24 [‡]
Male sex	68	26 (38)	36	19 (53)	0.15 [‡]
Weight (kg)	68	54.0 (17.5-79.9)	36	43.1 (20.5-94.3)	0.040 [‡]
BMI-for-age z score	67	−0.1 (−2.7 to 2.3)	36	−0.7 (−2.9 to 2.0)	0.11 [‡]
Laboratory					
CRP (mg/dL)	17	0.1 (0.1-0.4)	27	0.8 (0.1-7.4)	<0.001 [†]
ESR (mm/h)	37	6.0 (1.0-20.0)	28	10.5 (1.0-56.0)	<0.001 [†]
Albumin (g/dL)	44	4.6 (3.8-5.3)	29	3.9 (2.8-5.0)	<0.001 [†]
Adipokines					
Resistin (ng/mL)	64	12.6 (5.6-27.9)	36	21.1 (7.8-111.1)	<0.001 [†]
PAI-1 (ng/mL)	64	109.8 (15.10-1284.5)	36	170.2 (72.2-442.1)	<0.001 [†]
Cytokines					
HGF (pg/mL)	64	221.5 (70.1-849.0)	36	350.8 (88.5-2474.8)	0.001 [†]
TNF-α (pg/mL)	64	13.4 (2.1-276.7)	36	14.1 (2.6-348.0)	0.096 [†]
IL-1β (pg/mL)	64	2.2 (0.5-176.3)	35	7.7 (0.6-159.3)	0.016 [†]
IL-6 (pg/mL)	64	1.5 (0.5-115.5)	36	3.1 (0.7-56.3)	<0.001 [†]
Adipose tissue values					
VAT volume (cm³) MRI/CT	17	459 (153-945)	26	436 (107-2367)	0.50*
VAT:SAT ratio MRI/CT	13	0.29 (0.10-0.63)	20	0.41 (0.17-2.32)	0.15*

*P value adjusted for BMI-for-age z score in logistic regression model with log-transformed MRI/CT parameter.

†Wilcoxon rank sum test.

‡Pearson χ^2 test.

ESR indicates erythrocyte sedimentation rate.

gastroenterologist. The median time to escalation to biologics was 157 days (19-341 days), with 2 patients starting within 28 days of diagnosis; in addition, 73% were treated with immunomodulators before initiation of biologics. Two patients were lost to follow-up. The age, sex, BMI-for-age z score, and baseline sh-PCDAI were similar between the groups that received and did not receive biologics (Table 2). There was a statistically significant higher incidence of growth failure at baseline in the group that required biologics by 12 months ($P = 0.048$). The CRP was also significantly higher at baseline in patients with CD requiring biologics by 12 months than in those who did not require biologics (2.3 mg/dL vs 0.2 mg/dL; $P = 0.024$), with no additional differences in any other laboratory values at baseline.

There was no difference in the use of immunomodulators (thiopurines or methotrexate) between the biologic and nonbiologic groups (Table 2). Infliximab was the biologic used in 93% of patients with CD. The median time to initiation of biologic therapy was 22.4 weeks (2.7-48.7 weeks).

Among adipokines, baseline resistin levels in patients with CD who required biologic therapy were significantly higher than in those who did not require biologics (Table 2; 29.8 ng/mL vs

13.8 ng/mL; $P = 0.004$). None of the other adipokines or cytokines were significantly different at baseline among patients who went on to require biologic therapy compared with those who did not (Supplementary Table 2). The baseline VAT volumes were higher, though not with a statistically significant difference, in patients with CD who required biologics (628.8 cm³ vs 409.6 cm³; $P = 0.10$).

Nonparametric Spearman correlations of baseline adipokines and VAT volume in patients with CD are shown in Supplementary Table 4. Resistin had a statistically significant positive correlation with IL-6 ($P < 0.001$), HGF ($P = 0.004$), and CRP ($P = 0.001$) levels but no such correlation was found for PAI-1 or leptin. Whereas leptin had a statistically significant positive correlation with the BMI-for-age z score ($P < 0.001$) and the VAT volume ($P = 0.014$), resistin and PAI-1 did not. The VAT volume also positively correlated with the BMI-for-age z score ($P = 0.005$) and CRP ($P = 0.029$) but did not correlate with IL-6, HGF, or albumin.

Change in Baseline Anthropometrics, Laboratory Values, and Adipokines During Follow-up

Change in anthropometric measures and clinical laboratory values in patients with CD from baseline to the 6- and 12-month

TABLE 2. Characteristics of Pediatric Patients With CD Based on Exposure to Biologic Therapy by 12 Months

Factor	Total (n = 36) [†]	No Biologics (n = 19)		Biologics (n = 15)		P
	Median (minimum-maximum)/n (row %)	n	Median (minimum-maximum)/n (row %)	n	Median (minimum-maximum)/n (row %)	
Clinical factors at diagnosis						
Age	13.0 (7.0-18.0)	19	12.0 (8.0-18.0)	15	13.0 (7.0-17.0)	0.62 [‡]
Male sex	19 (53)	19	11 (58)	15	8 (42)	0.79 [‡]
BMI-for-age z score	-0.7 (−2.9 to 2.0)	19	−0.5 (−2.9 to 2.0)	15	−0.8 (−2.0,1.9)	0.58 [‡]
Growth failure	15 (42)	19	5 (36)	15	9 (64)	0.048 [‡]
sh-PCDAI		19		15		0.36 [‡]
Mild (10-25)	19 (53)		12 (67)		6 (33)	
Moderate (>25-40)	15 (42)		5 (36)		9 (64)	
Severe (>40)	2 (6)		2 (100)		0 (0)	
Hemoglobin (g/dL)	11.9 (7.4-16.3)	15	13.0 (10.2-16.3)	15	11.2 (7.4-15.7)	0.085 [‡]
CRP (mg/dL)	0.8 (0.1-7.4)	13	0.2 (0.1-4.3)	13	2.3 (0.1-7.4)	0.024 [‡]
ESR (mm/hr)	10.5 (1.0-56.0)	13	9.0 (1.0-32.0)	13	14.0 (3.0-56.0)	0.31 [‡]
Albumin (g/dL)	3.9 (2.8-5.0)	14	4.2 (2.8-5.0)	13	3.7 (3.2-4.6)	0.13 [‡]
Medical therapy by 12 months						
Thiopurine (azathioprine/mercaptopurine)	25 (74)	19	14 (56)	15	11 (44)	0.99 [¶]
Methotrexate	5 (15)	19	1 (20)	15	4 (80)	0.15 [¶]
Infliximab	14 (41)	19		15	14 (93)	<0.001 [‡]
Adalimumab	1 (3)	19		15	1 (7)	0.44 [¶]
Prednisone	32 (94)	19	18 (56)	15	14 (44)	0.99 [¶]
Adipokines at diagnosis						
Resistin (ng/mL)	21.1 (7.8-111.1)	19	13.8 (7.8-111.1)	15	29.8 (12.6-57.6)	0.004 [‡]
PAI-1 (ng/mL)	170.2 (72.2-442.1)	19	161.8 (72.2-264.4)	15	173.5 (82.5-442.1)	0.47 [‡]
Adipokines at 12 months						
Resistin (ng/mL)	8.9 (4.7-36.2)	9	9.9 (6.7-36.2)	14	8.5 (4.7-17.1)	0.18 [‡]
PAI-1 (ng/mL)	111.9 (0.0-270.7)	9	147.8 (53.8-270.7)	14	101.9 (0.0-208.0)	0.11 [‡]
Change in adipokines from baseline to 12 months						
Resistin (ng/mL)	−14.7 (−48.5 to 13.3)	9	−1.5 (−15.9 to 13.3)	14	−18.1 (−48.5 to −4.3)	0.002 [‡]
PAI-1 (ng/mL)	−58.3 (−297.9 to 129.4)	9	−2.3 (−99.9 to 129.4)	14	−69.3 (−297.9 to −5.8)	0.010 [‡]
VAT values at baseline						
VAT volume (cm ³) MRI/CT at baseline	447.7 (106.6-2366.6)	12	409.6 (106.6-1037.0)	12	628.8 (136.0-2366.6)	0.10*
VAT:SAT ratio MRI/CT at baseline	0.42 (0.17-2.32)	11	0.40 (0.17-0.69)	8	0.64 (0.17-2.32)	0.20*

*P value adjusted for BMI-for-age z score in logistic regression model with log-transformed MRI/CT parameter.

[†]Wilcoxon rank sum test.

[‡]Pearson χ^2 test.

[§]Fisher exact test.

ESR indicates erythrocyte sedimentation rate.

follow-ups are shown in [Table 2](#). Although there was improvement over time in the BMI-for-age z score, hemoglobin, CRP, erythrocyte sedimentation rate, and albumin in both the biologics and no-biologics groups, there was no statistically significant difference in the degree of improvement between the 2 groups.

Among adipokines, whereas there was a significantly greater reduction in resistin ($P = 0.002$) and PAI-1 ($P = 0.010$)

at the 12-month follow-up in patients on biologics compared to those not on biologics, there was no significant change in adiponectin or leptin levels between the 2 treatment groups ([Table 2](#), [Supplementary Table 2](#)). Similarly, there was a significantly greater reduction in HGF and IL-6 at 12-month follow-up in patients on biologics compared to those not on biologics but no significant changes in TNF- α , IL1- β , or IL-8

values. Only 6 patients had a CRP measured at 12 months, limiting the ability for meaningful analysis of this marker.

Baseline Resistin Values and Predicting Escalation to Biologics

There was no difference in the resistin levels at baseline in patients stratified by mild or moderate-severe clinical activity (as assessed by the sh-PCDAI) or the presence or absence of growth failure (Supplementary Table 5 and Supplementary Fig. 1). A model to predict escalation to biologic therapy (AUC analysis) showed that baseline resistin levels were superior (AUC, 0.82; 95% CI, 0.67-0.97) to the baseline sh-PCDAI values (AUC, 0.58; 95% CI, 0.41-0.76; $P = 0.009$). Baseline resistin levels were also numerically superior to baseline CRP values, but this difference was not statistically significant (AUC, 0.78; 95% CI, 0.59-0.97; $P = 0.69$; Fig. 2).

A baseline resistin level of ≥ 23.1 ng/mL at diagnosis predicted the escalation to biologics in the first year after diagnosis with a sensitivity of 73% and a specificity of 79%. A resistin level of ≥ 29.8 ng/mL had a specificity of 95% (sensitivity 53%; AUC, 0.82; $P = 0.015$ for model with log-scaled resistin) to predict escalation to biologics in the first year after diagnosis.

FIGURE 2. Resistin, sh-PCDAI, and CRP as predictors of treatment with biologics in pediatric CD. Receiver operator characteristic analysis performed for baseline resistin to determine the predictive capacity to predict escalation to biologics by 12-month follow-up as compared to sh-PCDAI and CRP. Resistin vs sh-PCDAI, $P = 0.009$; resistin vs CRP, $P = 0.69$.

DISCUSSION

In this prospective study of pediatric patients with CD, we evaluated serum adipokines and VAT volumes in newly diagnosed pediatric patients with CD and found significant differences not only compared to CP but, notably, also in patients with CD who required biologic therapy in the first 12 months after diagnosis compared with those who did not require biologic therapy.

Although there is increasing concern for the role of VAT in CD, there are few studies of adipokines and VAT in the pediatric CD population. We report several novel findings on the value of assessing adipokines in the context of VAT and clinical inflammatory activity. First, the levels of the adipokines resistin and PAI-1 were significantly higher in patients with CD at the time of CD diagnosis than in CP and were independent of VAT volume. Second, and most important, baseline resistin and CRP levels were significantly higher in those patients with CD who ultimately required initiation of biologic therapy than in those not requiring biologics in the first year after diagnosis. In particular, both resistin and CRP had better predictive value for escalation to biologics than did other baseline clinical and laboratory parameters and the sh-PCDAI. In addition, 3 patients requiring biologics had a CRP of ≤ 1.0 mg/dL but resistin > 29 ng/mL at diagnosis; thus, in these patients, the baseline CRP levels did not perform as well as the baseline resistin as a predictive factor. It is possible that these patients have a genetic variant in the CRP gene that precludes the expected CRP elevation and that resistin is a better-suited biomarker.²⁵ Third, resistin and PAI-1 levels significantly improved at 12 months in patients with CD treated with biologics compared with patients who did not receive biologics. Taken together, these results suggest the utility of resistin measurement as a predictive biomarker in early pediatric CD along with PAI-1 as a complementary biomarker.

The potential pathogenic role of VAT was initially considered after researchers observed mesenteric fat wrapping around inflamed regions of intestine at the time of intestinal resection.⁶ Coffey et al²⁶ reported that patients in whom the mesenteric fat was excised during ileocecal resection had a reoperation rate of 2.9% at a mean follow-up of 4 years compared with 40% ($P = 0.003$) for those in whom mesenteric fat was retained, suggesting that VAT significantly contributes to the transmural inflammation of CD. Previous research has shown that higher VAT volume in pediatric patients with IBD is associated with worse disease-related outcomes.⁹ In addition, Frivolt, Hetterich, et al²⁷ reported that pediatric patients with CD had increased VAT volumes as measured by MRI compared with CP and that the VAT volume correlated with the duration of disease, which may account for the deficit of increased VAT volume in patients with CD at diagnosis compared with CP at the time of radiologic assessment in our study. In adults with CD, the VAT-to-SAT ratio but not total VAT was reported

to be associated with a progressive disease course.^{28, 29} In our study, the total VAT volume in those requiring biologics and the VAT-to-SAT ratios in patients with CD compared to CP were numerically higher, with a trend toward statistical significance that would likely be statistically significant with a larger sample size.

The volume of adipose tissue alone may also underestimate the inflammatory role of VAT in the catabolic state of pediatric CD, and our study supports the notion that VAT biological activity rather than its volume per se is a superior marker of inflammation because resistin emerged as a strong predictor of escalation to biologic therapy and during clinical follow-up. Furthermore, we did not reassess VAT volume at 1 year after diagnosis because of the design of our study, but if we had, then the VAT volume may have shown a different correlation with disease activity or adipokine levels.

Resistin and PAI-1 are adipokines with well-recognized immune and proinflammatory functions and have previously been reported to be involved in IBD.^{30, 31} Resistin is secreted by macrophages within VAT and is associated with the activation of the innate immune inflammatory response by stimulating the secretion of IL-6, IL-12, and TNF- α .³² In 2 studies,^{33, 34} resistin levels were elevated in adult patients with IBD compared with CP; the second study, a follow-up study by Karmiris et al,³⁴ reported a significant decrease in resistin levels in patients with IBD after treatment with infliximab.^{33, 34} A recent study by Frivolt, Schwerdt, et al³⁵ also reported higher resistin levels at diagnosis that decreased after infliximab treatment over 14 weeks in pediatric CD ($n = 18$). Similarly, we found significantly greater reductions in resistin ($P = 0.002$) and PAI-1 ($P = 0.010$) levels at 12 months among patients on biologics compared with those not on biologics. The adipokine PAI-1 inhibits tissue-type and urokinase-type plasminogen activators, resulting in enhanced systemic clot formation that may promote the vasculitis seen in the intestinal wall in IBD.³⁶ Adult patients with IBD have been shown to have increased levels of PAI-1 compared with CP, and a 4G/4G genotype of PAI-1 was predictive of penetrating disease behavior in adults with CD.^{31, 37} In addition, Kaiko et al³⁸ reported an increase in the gene expression of *SERPINE1* (encodes PAI-1) and in PAI-1 expression using immunofluorescence in colonic epithelial cells from intestinal biopsies of patients with active CD refractory to anti-TNF- α therapy.^{38, 39} The production of PAI-1 by epithelial cells in addition to VAT also offers an alternative explanation as to why the VAT volume is not significantly expanded in those with active CD.³⁸ Although this finding is suggestive of a significant role of adipokines in IBD, there are still only a limited number of studies evaluating their role, and ours is the first to evaluate adipokines as a biomarker predictive of escalation to biologic therapy.

The major strengths of our study are the prospective evaluation of adipokine levels in treatment-naïve, newly

diagnosed pediatric patients with CD with a predominantly inflammatory phenotype at the time of diagnosis and at the 12-month follow-up of those patients. Limitations include the recruitment of patients with relatively low disease severity as evidenced by a mild sh-PCDAI score in more than half of the patients. Notably, the patient with the highest resistin level of 111.1 ng/mL was recommended to receive biologic therapy early after diagnosis but declined and was thus analyzed in the nonbiologic group, although biologic therapy was initiated 3 years later without significant complication. The 6 patients with a mild sh-PCDAI at diagnosis who escalated to biologic therapy had similar baseline resistin levels (median resistin of 22.3 ng/mL), CRP levels, and presence of growth failure ($n = 4$) compared with those with a moderate-severe sh-PCDAI at diagnosis but were not escalated to biologics (Supplementary Table 5). In addition, both patients with a severe sh-PCDAI at diagnosis were not escalated to biologics. Both of these outcomes highlight the significant limitations of disease activity indexes based on clinical symptoms to accurately measure the degree of disease activity and the importance of assessing growth failure in pediatric CD, and to assess long-term prognosis. Objective biomarkers such as resistin would be better utilized as markers of disease activity rather than as patient-reported symptoms.

The decision to escalate treatment to biologics was at the discretion of the treating provider and was not a standard set by the study. However, 7 different providers started biologics on 15 patients, limiting the provider-treatment-preference bias. Finally, we note that 27% of pediatric patients with CD did not have imaging at the time of diagnosis.

There are also possible variations in the VAT measurement between imaging modalities (MRE vs CTE). The VAT measurements were conducted using 2 different methods because of the inherent differences in the captured images and the expertise required to annotate the VAT, which may have led to discrepancies in the VAT measurements. When comparing the analysis of MRE versus CTE, we did not find a significant difference in the VAT volumes that could not be accounted for by age, BMI, or disease activity.

Although larger studies are needed, our findings suggest that patients with a resistin level >23 ng/mL at diagnosis should be closely monitored for continued disease activity and considered for early biologic initiation. There is further work to be done as we evaluate the validity of noninvasive markers of disease activity and progression in pediatric CD. Our study builds on previous work in adult IBD evaluating the potential role of VAT biomarkers in CD. Although it remains unknown whether abnormal adipose tissue contributes to the underlying development of CD or is a risk factor for prolonging inflammation once the disease develops, there should be consideration for therapy applied specifically to the adipokine inflammatory response in CD.

CONCLUSIONS

In this prospective study of newly diagnosed pediatric patients with CD, we found that the adipokine resistin was significantly higher in patients with CD than in CP and along with CRP was strongly predictive of escalation to biologics in the first year after CD diagnosis, independent of VAT volume. In addition, resistin and PAI-1 levels significantly improved in patients with CD after treatment of biologics compared with those not on biologics. These results suggest the utility of resistin as a predictive biomarker in pediatric CD in addition to the already established CRP inflammatory marker. Further studies evaluating resistin and PAI-1 should be conducted to evaluate their utility as biomarkers superior to current conventional assessments.

SUPPLEMENTARY DATA

Supplementary data are available at *Inflammatory Bowel Diseases* online.

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