

## Review Article

# The role of chemokines and adipokines as biomarkers of Crohn's disease activity: a systematic review of the literature

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**Abstract:** Introduction: Crohn's disease (CD) is an inflammatory bowel disease (IBD) that affects the gastrointestinal tract and can have a major impact on the patient's quality of life and social/professional activities. Asymptomatic patients, or those with mild symptoms, experience the active disease with subclinical manifestation. Systematic review (SR) was performed to look for evidence for the role of chemokines and adipokines as markers for CD activity. Methods: This SR was conducted by searching published studies in international and regional databases up till July, 2020. CD patients were adults with the disease in activity or remission. All adipokines and chemokines were considered for the analysis and the Rayyan QCRI system was used. Results: In total, 20 studies were included. Six addressed chemokines and eight adipokines as potential biomarkers of CD activity. CXCL8 was the most studied chemokine (8 studies) and the results were controversial, with 62.5% showing a significant association with CD activity. CXCL10 was investigated by 4 studies and 50% identified it as a potential biomarker. CCL2, CCL11, CCL26 and CXCL1 were examined by 2 articles each. CXCL8 ( $P=0.002/P=0.001$ ) and CXCL1 ( $P<0.001$ ) presented the lowest?  $P$  value, which qualifies them as potential markers of disease activity. All the adipokines were tested in peripheral blood but 44.4% were also tested in intestinal mucosa, while the percentage in the chemokines' studies was 76.9% in peripheral blood, 46.1% in intestinal mucosa and 7.6% in urine sample respectively. Conclusion: The development of disease activity biomarkers for CD is becoming relevant for clinical practice. Chemokines and adipokines have the potential to signalize CD activity, but validation in larger cohorts of patients, preferable multicenter studies are still needed.

**Keywords:** Crohn's disease, inflammatory bowel disease, chemokine, adipokine, inflammation

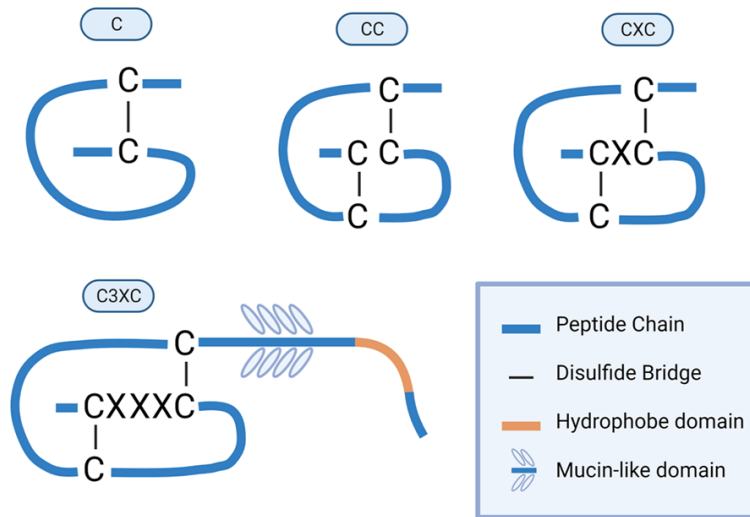
## Introduction

Crohn's disease (CD) is an inflammatory bowel disease (IBD) that can affect the entire gastrointestinal tract and its pathophysiology is associated with an exacerbated immune response. It usually impacts the patient's quality of life, professional activities and the psychosocial environment. The diagnosis is more frequent among young individuals and the prevalence has been increasing in several developing countries. In spite of the relatively low mortality rate, CD's high morbidity makes it a current public health problem [1].

CD is characterized by segments of transmural inflammation that can affect any part of the

gastrointestinal tract, from the mouth to the anus, with a greater involvement of the terminal ileum and the proximal colon. Its endoscopic appearance is one of compound deep and ser-piginous ulcers, and affects areas interspersed with normal mucosa. Non-caseous epithelioid granuloma is a specific histological marker, but it is infrequent [2]. Exacerbation and remission phases are alternated. Therefore, CD patients must be monitored regularly to avoid permanent bowel damage. It is worth mentioning that asymptomatic patients or those with mild symptoms should have the disease's activity confirmed by complementary examinations. These cases with subclinical manifestation require intervention to change the course of the disease [3].

**Chemokines Classes**



**Figure 1.** Classes of chemokines which have been defined by the arrangement of the conserved cysteine (C) residues of the mature proteins.

The diagnosis is based on clinical symptoms, the most frequent of which are fever, weight loss, anemia, abdominal pain, diarrhoea, digestive hemorrhage, extra intestinal manifestations such as arthritis, uveitis and erythema nodosum, in addition to ileocolonoscopy with biopsy and/or entero magnetic resonance imaging (MRI) [4]. Patients are monitored with clinical evaluation and complementary examinations, including endoscopy with biopsies and/or imaging and analysis of serum and fecal biomarkers. Serum C-reactive protein (CRP) and fecal calprotectin levels are markers of IBD activity, but they do not always correlate with the degree of intestinal mucosa inflammation observed during colonoscopy examination. Thus, new biomarkers are urgently needed in order to more accurately predict mucosal damage [5]. The most common tissues of which biomarkers have been evaluated are intestinal mucosa, adipose tissue, and peripheral blood of the CD patients.

The inflammatory response in CD patients is mainly based on the increasing expression of cytokines and chemokines. Cytokines are defined as a group of proteins released by the immune system that regulate the migration of interleukins and lymphocytes to the inflamed area, acting as mediators of the immune response. On the other hand, chemokines (Greek-kinos, movement) are a family of small

cytokines, or signaling proteins secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells. They are chemotactic cytokines [6].

Cytokines are classified as chemokines according to their behavior and structural characteristics. In addition to being known for mediating chemotaxis, all chemokines are approximately 8-10 kilodaltons in mass unit and have four cysteine residues in conserved locations that are key to form their 3-dimensional shape (Figure 1). These proteins have historically been known as the SIS family of cytokines, SIG family of cytokines, SCY family of cytokines, *Platelet factor-4* superfamily or *intercrines*. Some chemokines are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to a site of infection, while others are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development [7]. Chemokines have been classified into four main subfamilies: CXC, CC, CX3C and XC. All of these proteins exert their biological effects by interacting with G protein-linked transmembrane receptors called chemokine receptors, which are selectively found on the surfaces of their target cells [7-9].

At the same time, the literature emphasizes the hypothetical role of mesenterial adipose tissue in the pathophysiology and CD activity [10-12]. Therefore, the traditional view of adipose tissue as a passive reservoir for energy storage is no longer valid, because recent studies have begun to regard it as an endocrine organ and have shown that it secretes relevant inflammatory factors [7, 11-13]. These factors, also called adipokines, are cytokines (cell signaling proteins) secreted by adipose tissue. The first adipokine to be discovered was leptin in 1994 [13]. Since that time, hundreds of adipokines have been described [14]. They were identified and classified based on the processes being controlled. These processes

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include lipid homeostasis, immune function, insulin sensitivity, blood pressure control, homeostasis, appetite and energy balance [15-17]. Some studies consider the possibility that CD's inflammatory process is due to the impaired function of the adipokine secretion [18-20].

Rodrigues et al. demonstrate that patients without clinical and endoscopic disease activity show inflammation of the mesenteric adipose tissue near the intestinal affected area observed in surgical specimens [21]. Moreover, CD patients with active disease showed decreased levels of serum adiponectin, an anti-inflammatory adipokine, when compared to those in remission and the control group, which suggests a dysregulation of an anti-inflammatory pathway to counterbalance the inflammation. There was no difference in serum and tissue leptin levels, which were similar among the groups [21].

The role of biomarkers for CD activity is to reduce the time spent to detect disease activity and the need for invasive procedures to access disease activity (such as colonoscopy). In addition, it also enables clinicians to anticipate changes in the therapeutic strategy, and thereby avoid serious complications such as perforation, intestinal occlusion, fistulas and abscesses. The ability to predict the behavior and course of the disease, as well as to identify patients at risk of rapid progression towards complications, can avert the use of invasive resources and reduce the economic impact of this disease.

There is much less research on the impact of chemokine's and adipokine's role in the inflammatory process of Crohn's disease than on inflammatory factors such as cytokines or other secreted proteins. Chemokines and adipokines can be secreted by several types of cells, and they may play a relevant role in the maintenance of the inflammation in CD, either chemoattracting immune cells to the affected intestinal area (chemokines), or displaying pro- and anti-inflammatory properties which can be secreted by adipose cells from the affected mesenteric adipose tissue in CD (adipokines). Given the above, we performed a systematic review (SR) looking evidence at the literature for the role of chemokines and adipokines as markers for CD activity, with a view towards future application in clinical practice.

## Method

### *Study design*

The SR dealt with the comparison of all the empirical evidence that fits the specified eligibility criteria, to answer a specific question: "Chemokines and adipokines as activity markers of Crohn's disease". This analysis went on to follow some fundamental stages: definition of the research question, the databases, the search interval, the details of the search elements, the descriptors, extensive and ordered search in the databases, the inclusion and exclusion criteria, the collection of essential data, the choice of articles, assessment of eligibility and the application of exclusion criteria [22, 23]. The selected articles were read and those with inconsistencies were removed from the SR. Afterwards, the analysis of results and the discussion of the study with the evidence were performed. This SR is in line with the recommendation "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA), described in a review in 2015 [24].

### *Research strategy, screening and data extraction*

To choose the study and export the data, the Rayyan QCRI system was used. Rayyan is software developed by the Qatar Computing Research Institute (QCRI), a tool to assist in archiving, organizing and selecting articles [25].

Publications from the years 2002 to 2020 were included in the study. The final search in the selected databases was carried out on July 20, 2020, with 344 articles identified in English, Spanish and Portuguese languages.

The selection of articles published in the Rayyan system was carried out by two reviewers, autonomously and independently, and a third reviewer was responsible for analysing and deciding on the inclusion or exclusion of the article, especially in relation to those who presented a contradictory and conflicting decision.

The search for articles was carried out in the databases PUBMED, PUBMED PM C, BVS-BIREME, SCOPUS, WEB OF SCIENCE, EMBASE, COCHRANE, EBSCOHOST, PROQUEST and ENDNOTE WEB, which are shown in **Table 1**.

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**Table 1.** Databases and the descriptors used for the systematic review

DATABASE	Vocabulary of subjects	Descriptors and free terms used in the search strategy				
		1	2	3	4	5
PUBMED	Mesh	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation
	Medical Subject Headings					
PUBMED PMC	Mesh	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation
	Medical Subject Headings					
BVS/BIREME	DeCS	"Crohn's Disease" "Enfermedad de Crohn"	Biomarcadores	Chemokines	Adipoquinas	Inflamación
		"Doença de Crohn"	Biomarcadores	Quimiocinas	Adipocinas	Inflamação
EBSCOHORT	Mesh	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation
SCOPUS	Mesh	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation
WEB OF SCIENCE	Mesh	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation
EMBASE	Emtree	"Crohn's Disease"	Biomarkers	Chemokines	Adipokines	Inflammation

**Table 2.** Population, interventions, comparators and outcome parameters used for the systematic review

Population	Crohn's disease patients
Interventions	Studies demonstrate the population of Crohn's disease patient with a disease activity inferred by the expression of chemokines and/or adipokines
Comparators	Chemokines and/or adipokines with disease activity
Outcome	Studies demonstrate the population of Crohn's disease patient with a disease activity inferred by the expression of chemokines and/or adipokines

## Inclusion criteria

The keywords "Crohn's disease", "chemokines", "adipokines", "biomarkers" and "inflammation" were chosen after reading material related to the researched topic. To expand and guide the search, an association of the descriptors was carried out by means of Boolean operators "OR" and "AND". The Boolean operators "OR" and "AND" were used to add or restrict the search, as follows: "Crohn Disease AND Biomarkers AND (Chemokines OR Adipokines) AND Inflammation".

Furthermore, this type of review is defined by the type of population (participants), types of interventions (and comparisons) and types of implications. The acronym PICO (population, interventions, comparators and outcome) has been widely used in this routine. In this research, the English acronym "PICO" was used as the study design. Thus, **Table 2** summarizes the main aspects of interest for the SR.

Reviews, SRs and meta-analyses that did not show original data were excluded from this review. Moreover, we considered only studies with human samples. Therefore, animal studies were excluded.

## Results

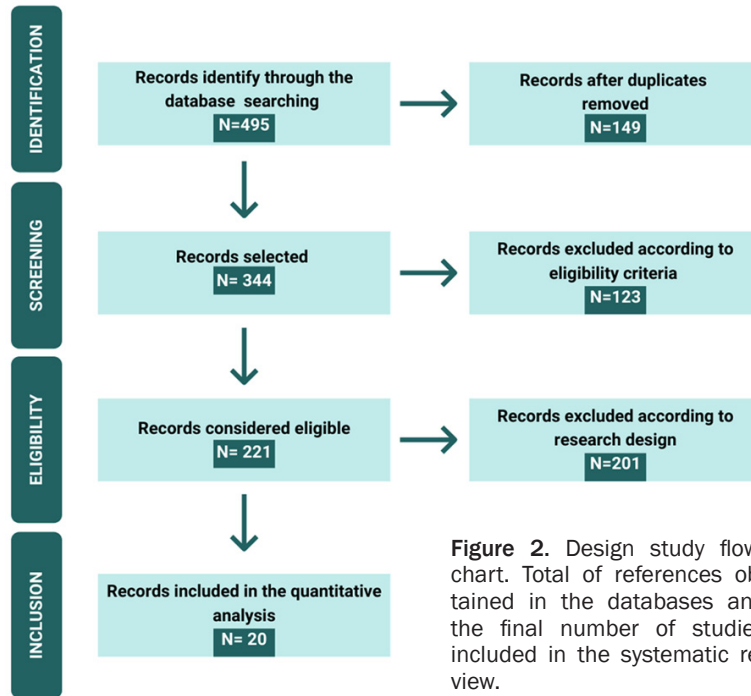
### Literature search and study characteristics

The thorough search process carried out in nine databases until July 28th, 2020, resulted in the immediate retrieval of 495 potentially relevant references in the period from 2002 to 2020. The articles' collection was made possible by the library staff of the School of Medical Sciences (SMS) of the University of Campinas (Unicamp), who provided support in the search and identification of the references. After reviewing the articles, 475 were excluded for being duplicates, for not meeting the eligibility criteria, and were excluded according to our research design. Finally, this SR included twenty studies. **Figure 2** shows the flowchart of the references' selection.

The chosen references included in the SR are shown in the following **Tables 3, 4**.

We evaluated several aspects of each study including the type of chemokine/adipokine investigated, the subjects, origin of the samples (peripheral blood, intestinal biopsies, urine), year of publication, and criteria for assessing disease activity and judging whether the chemokine/adipokine evaluation showed sig-

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**Figure 2.** Design study flow-chart. Total of references obtained in the databases and the final number of studies included in the systematic review.

nificant results (**Tables 1 and 2**). **Figure 3** illustrates the main variables obtained from each study included in the SR.

### *Chemokines and adipokines as significant markers for Crohn's disease activity*

Among the twenty selected articles, six (30%) addressed only chemokines [2, 26-30] and eight (40%) only adipokines [21, 31-37] as potential biomarkers for CD activity. Six references mentioned both types of cytokines [38-43]. The samples were obtained from peripheral blood (60%), and intestinal mucosa (40%); one study evaluated urine samples (5%). This latter one analyzed peripheral blood and intestinal mucosa simultaneously. The assessment of CD activity was heterogeneous, with endoscopic index (40%), clinical index (40%), calprotectin level (10%), and histologic parameters (10%) as the main criteria.

Among the adipokines, all were tested in peripheral blood, and 44.4% were also tested in the intestinal mucosa. Adiponectin, leptin, IL-6, and TNF- $\alpha$  were tested in more than one type of sample, like peripheral blood and intestinal mucosa. While the percentage among the chemokine's studies was 76.9% in peripheral blood, 46.1% intestinal mucosa, and 7.6% in urine sample. CCL2, CXCL8, and CXCL10 were

tested in peripheral blood and intestinal mucosa in different studies.

CXCL8 was the most studied chemokine (eight studies), and the results were controversial, with 62.5% (five studies) showing a significant association with CD activity ( $P=0.002$ ;  $P<0.05$ ;  $P<0.05$ ;  $P<0.05$ ;  $P=0.001$  respectively) [2, 27, 28, 30, 42]. Four researchers group explored CXCL10 and 50% identified it as a potential biomarker ( $P<0.05$ ) [40, 42]. CCL11 and CCL26 were considered by two articles each and they did not show significant results [38, 40]. CCL2 was explored in two studies, one of them positively correlated with disease activity ( $P<0.05$ ) [40].

CXCL1 was included in two studies as well, with significant results in both ( $P=0.001$ ;  $P<0.05$ ) [26, 41]. Only three references studied CX3CL1, CCL20 and CXCL3 separately, with positive correlation with the adopted inflammation criteria ( $P=0.02$ ;  $P<0.05$ ;  $P<0.05$ , respectively) [27, 33, 34]. CCL3, CCL4, CCL17, and CCL22 were explored in one article each as well, but without significant results [38].

There were more studies that addressed adipokines than chemokines. IL-6 was studied in six studies. All of them showed it as a potential inflammation biomarker in CD ( $P<0.05$ ;  $P<0.01$ ;  $P<0.05$ ;  $P<0.05$ ;  $P<0.05$ ;  $P=0.004$ , respectively) [38-43]. Five studies explored adiponectin levels, while there was only one with a significant result ( $P<0.01$ ), when comparing active CD with healthy controls [21, 31-34]. Resistin and leptin were investigated in another five works. Resistin presented 60% (three) of the studies which demonstrated significant  $P$ -value ( $P=0.04$ ;  $P=0.02$ ;  $P=0.014$ , respectively) [31, 33, 36], while leptin presented only one with a significant result ( $P<0.01$ ) [35]. All of four references on TNF- $\alpha$  showed it as a potential activity biomarker for CD ( $P<0.05$ ;  $P<0.05$ ;  $P<0.05$ ;  $P<0.002$ ) [39, 40, 42, 43]. One study explored omentin and confirmed a  $p$  of significance equal to 0.012 [37]. Ghrelin was evalu-



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**Table 3.** Scientific articles about chemokines as biomarker of CD activity included in the systematic review

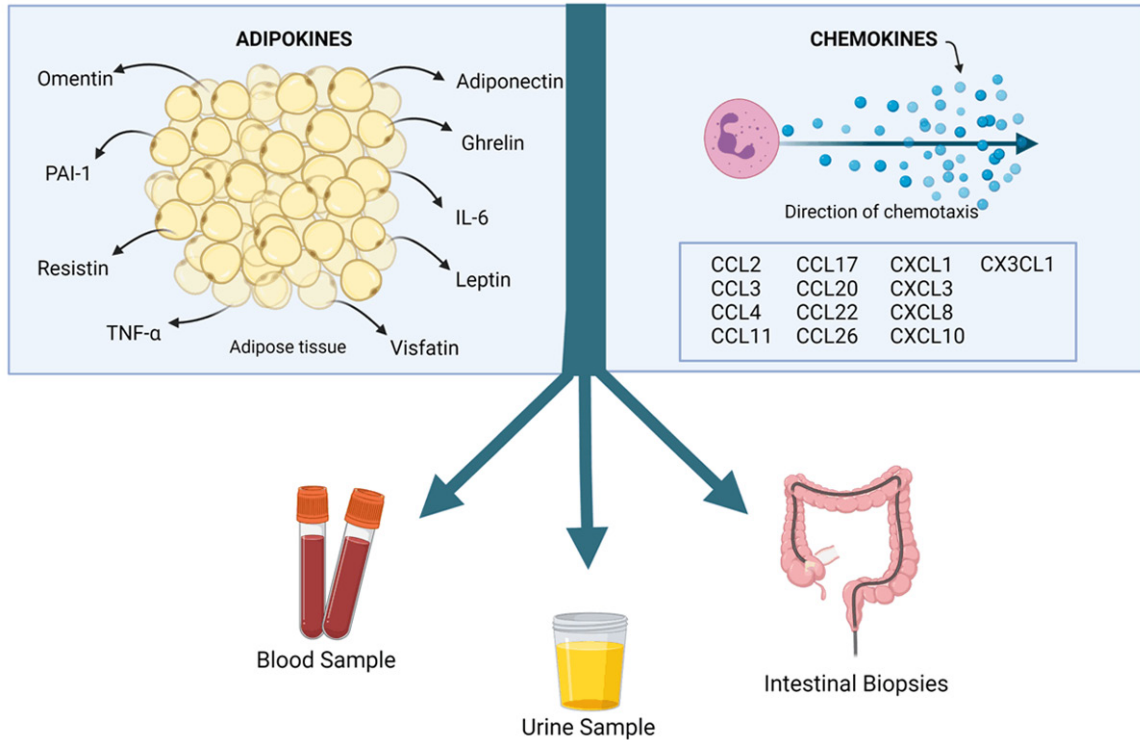
CHEMOKINE	ORIGINAL NAME	RECEPTOR	GENE	ARTICLE	SAMPLES	YEAR	CRITERIA FOR DISEASE ACTIVITY	IS IT A POTENTIAL BIOMARKER?
CCL2	MCP-1	CCR2	Scya2	Bourgonje et al./Christophi et al.	Peripheral Blood/Intestinal Mucosa	2018/2012	Calprotectin level/Histologic	NO/YES (p<0.05)
CCL3	MIP-1 $\alpha$	CCR1/CCR5	Scya3	Bourgonje et al.	Peripheral Blood	2018	Calprotectin level	NO
CCL4	MIP-1 $\beta$	CCR5	Scya4	Bourgonje et al.	Peripheral Blood	2018	Calprotectin level	NO
CCL11	Eotaxin-1	CCR3	Scya11	Bourgonje et al./Bourgonje et al.	Peripheral Blood/Peripheral Blood	2018/2019	Calprotectin level/Endoscopic Index	NO/NO
CCL17	TARC	CCR4	Scya17	Bourgonje et al.	Peripheral Blood	2018	Calprotectin level	NO
CCL20	LARC/Exodus-1	CCR6	Scya20	Christophi et al.	Peripheral Blood	2012	Histologic	YES (p<0.05)
CCL22	MDC	CCR4	Scya22	Bourgonje et al.	Peripheral Blood	2018	Calprotectin level	NO
CCL26	Eotaxin-3	CCR3	Scya26	Bourgonje et al./Bourgonje et al.	Peripheral Blood	2018/2019	Calprotectin level/Endoscopic Index	NO/NO
CXCL1	GRO $\alpha$	CXCR2	Scyb1	Hong et al./Leal et al.	Intestinal Mucosa/Intestinal Mucosa	2017/2014	Endoscopic Index/Endoscopic Index	YES (p<0.001)/YES (p<0.05)
CXCL3	GRO3, MIP-2 $\beta$	CXCR2	Scyb2	Leal et al.	Intestinal Mucosa	2014	Endoscopic Index	YES (p<0.05)
CXCL8	IL-8	CXCR1 CXCR2	Scyb8	Arsenescu et al./Boland et al. Bourgonje et al./Brand et al. Bruno et al./Christophi et al. Taha et al./Yarur et al.	Intestinal Mucosa/Intestinal Mucosa Peripheral Blood/Intestinal Mucosa Intestinal Mucosa/Intestinal Mucosa Urine/Peripheral Blood	2008/2015 2019/2006 2015/2012 2002/2016	Endoscopic Index/Endoscopic Index Endoscopic Index/Endoscopic Index Endoscopic Index/Histologic Clinical Index/Histologic	YES (p=0.002)/YES (p<0.05) YES (p<0.05)/YES (p<0.05) NO/NO YES (p=0.001)/NO
CXCL10	IP-10	CXCR3	Scyb10	Boland et al./Bourgonje et al. Christophi et al./Grip et al.	Intestinal Mucosa/Peripheral Blood Intestinal Mucosa/Peripheral Blood	2015/2018 2012/2009	Endoscopic Index/Calprotectin level Histologic/Calprotectin Level	YES (p<0.05)/NO YES (p<0.05)/NO
CX3CL1	Fractalkine	CX3CR1	Scyd1	Brand et al.	Intestinal Mucosa	2006	Endoscopic Index	YES (p=0.02)

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**Table 4.** Scientific articles about adipokines as biomarker of CD activity included in the systematic review

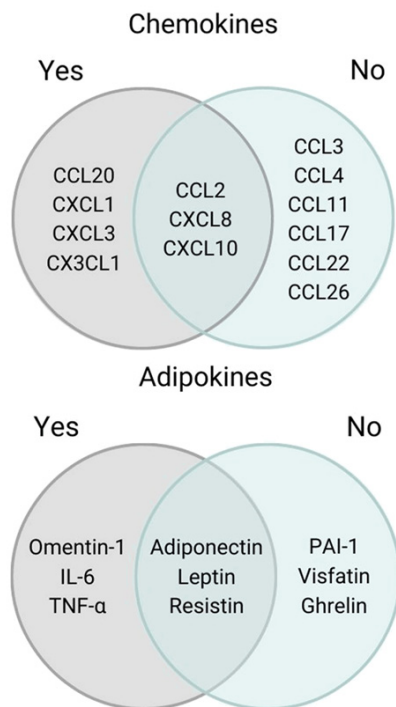
ADIPOKINE	ARTICLE	SAMPLES	YEAR	CRITERIA FOR DISEASE ACTIVITY	IS IT A POTENTIAL BIO-MARKER?
ADIPONECTIN	Karahman et al./Karmiris et al./Karmiris et al./Rodrigues et al./Theocharidou et al.	Peripheral Blood/Peripheral Blood/ Peripheral Blood/Mesenteric Biopsies/ Peripheral Blood	2017/2007/2006 2012/2016	Clinical Index/Clinical Index/Clinical Index/ Endoscopic Index/Clinical Index	NO/NO/NO YES (p<0.01)/NO
LEPTIN	Karahman et al./Karmiris et al./Karmiris et al. Rodrigues et al./Trejo-Vazquez et al.	Peripheral Blood/Peripheral Blood/ Peripheral Blood/Mesenteric Biopsies/ Peripheral Blood	2017/2007/2006 2012/2017	Clinical Index/Clinical Index/Clinical Index/ Endoscopic Index/Endoscopic Index	NO/NO/NO NO/YES (p<0.001)
OMENTIN-1	Yin et al.	Peripheral Blood	2014	Clinical Index	YES (p=0.012)
PAI-1	Trejo-Vazquez et al.	Peripheral Blood	2017	Endoscopic Index	NO
RESISTIN	Karmiris et al./Karmiris et al./Konrad et al. Theocharidou et al./Trejo-Vazquez et al.	Peripheral Blood/Peripheral Blood/ Peripheral Blood/Peripheral Blood/ Peripheral Blood	2007/2006/2007 2016/2017	Clinical Index/Clinical Index/Clinical Index/ Clinical Index/Endoscopic Index	YES (p=0.004)/NO/YES (p=0.02) YES (p=0.014)/NO
VISFATIN	Trejo-Vazquez et al.	Peripheral Blood	2007	Endoscopic Index	NO
GHRELIN	Karmiris et al./Trejo-Vazquez et al.	Peripheral Blood/Peripheral Blood	2006/2017	Clinical Index/Endoscopic Index	NO/NO
IL-6	Boland et al./Bourgonje et al. Bourgonje et al./Christophi et al. Leal et al./Yarur et al.	Intestinal Mucosa/Peripheral Blood Peripheral Blood/Intestinal Mucosa Intestinal Mucosa/Peripheral Blood	2015/2017 2019/2012 2014/2016	Endoscopic Index/Calprotectin Level Endoscopic Index/Histologic Endoscopic Index/Histologic	YES (p<0.05)/YES (p<0.01) YES (p<0.05)/YES (p<0.05) YES (p<0.05)/YES (p=0.004)
TNF- $\alpha$	Boland et al./Bourgonje et al. Christophi et al./Yarur et al.	Intestinal Mucosa/Peripheral Blood Intestinal Mucosa/Peripheral Blood	2015/2019 2012/2016	Endoscopic Index/Endoscopic Index Histologic/Histologic	YES (p<0.05)/YES (p<0.05) YES (p<0.05)/YES (p<0.002)

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**Figure 3.** Illustrated chart of the systematic reviews' aims, and the samples which most of the selected references worked with to correlate the chemokines and adipokine levels with Crohn's disease activity.

### Potential biomarkers of disease activity in Crohn's disease



**Figure 4.** Chemokines and adipokines significantly modulated in Crohn's disease according to the stud-

ies included in the systematic review. Potential biomarkers of disease activity are shown in gray in the Venn diagram.

ated in two studies, PAI-1 and visfatin in one; all of them revealed no significant results [31, 35]. **Figure 4** shows the potential biomarkers for active Crohn's disease.

### Discussion

This SR aims to explore what can be found in the literature about chemokines and adipokines as CD activity markers. The analysis began with an examination of convergence and divergence in the results. All studies dealt with a population of CD patients with and without activity of the disease defined by clinical index, endoscopic, radiological aspects, or calprotectin level. Therefore, the following topics were based on the studies which pointed towards chemokines and adipokines as possible markers of CD activity.

The chemokines were considered by their acronym (CCL, CXCL, CX3CL) or their "original names". Some articles conducted studies based on the chemokine receptors. We standardized



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the chemokine description by using acronyms below.

### *CCL20*

CCL20 is a small cytokine belonging to the CC chemokine family. It is strongly chemotactic for lymphocytes and weakly attracts neutrophils. Christophi et al. analyzed human intestinal mucosa and confirmed through RT-PCR that there is an increase of CCL20 levels 10 times more significant, and a higher CCL20/CCR6 ratio in CD patients with active disease compared to those in remission, suggesting a possible marker for CD activity [40].

### *CXCL10*

It is an 8.7 kDa protein, encoded by the CXCL10 gene and is secreted by several cell types in response to IFN- $\gamma$ . Boland et al., using biopsies from ileum and colon segments, showed increased mRNA CXCL10 expression in CD patients with active disease when compared to those in remission, suggesting this chemokine as a potential biomarker [42]. This result was supported by another study, which found increased levels of CXCL10 in CD inflamed colonic biopsies [42]. However, other two studies did not find a relation between CXCL10 level and fecal calprotectin in active CD patients [29, 39].

### *CXCL3*

Chemokine (C-X-C motif) ligand 3 was studied by Leal et al., who analyzed potential biomarkers for anti-TNF- $\alpha$  therapy response in the intestinal mucosa. They showed an upregulation of CXCL1 and CXCL3 chemokines, besides IL1B, S100A8, S100A9, SLC64A14, FCG3B (CD16b) and IL17A transcriptional levels in CD patients with active disease, regardless of the anti-TNF therapy use, suggesting these transcripts as markers of inflammation in CD [41].

### *CCL3, CCL4, CCL17, CCL22 and CCL26*

Another group of chemokines was depicted in a serum cytokine study (IL-6, CCL2, CCL3, CCL4, CCL11, CCL17, CCL22, CCL26, SAA, IL-17A, TNF- $\beta$  and others), and a correlation with CRP and fecal calprotectin was performed. Only IL-6, IFN- $\gamma$ , IL17A, and TNF- $\beta$  showed a significant association with these established serum biomarkers of disease activity [38, 39].

### *CXCL1*

CXCL1 is a small peptide belonging to the CXC chemokine family that acts as a relevant chemoattractant for several immune cells. Hong et al. and Leal et al. noticed an increased CXCL1 level in CD inflamed intestinal mucosa compared to CD in remission. Interestingly, intestinal mucosa of CD in remission still presented higher CXCL1 levels compared to healthy controls without CD. Moreover, CXCL1 was associated better with disease activity than CRP [26, 41].

### *CXCL8 and CCL11*

CXCL8 is the main mediator of the initial innate immune response to intracellular microbes and higher levels were found in UC patients, but not in CD patients. The samples were obtained from visibly inflamed colonic mucosa biopsies and were compared to paired samples from non-inflamed colonic mucosa from the same individuals [2, 27, 28, 40, 42].

The endoscopic criteria are still the standard for determining CD activity, despite of the high sensibility and specificity of the calprotectin test, which is a less invasive method. Thus, in a recent study, chemokines levels were compared with endoscopy disease activity. The SAA, IL-6, IL-8, and CCL11 levels in combination showed a reliable prediction for endoscopy inflammation activity in CD and can be important for monitoring active disease [39]. CCL11, CXCL8, SAA, IL17A, and TNF- $\alpha$ , individually, showed better predictive performances compared to CRP, fecal calprotectin and clinical scores, and they were equivalent to endoscopic parameters of disease activity [39].

### *CX3CL1*

There was a significant increase of CX3CL1 transcript level in inflamed lesions of CD patients when compared to the non-inflamed colonic mucosa, suggesting it as a marker of disease activity [27].

Concerning the studies which discussed the adipokines and their association or not with CD activity, there is the following.

### *Leptin and adiponectin*

The adipokines called leptin and adiponectin were mentioned in five articles, all with human

populations and blood sample analyzes. Karmiris et al., in two publications, evaluated the circulating levels of leptin, adiponectin and resistin in IBD patients, and analyzed the correlation between adipokines and treatment with infliximab. They found a possible association between adipokines and disease activity, but the changes in serum levels of the examined adipokines were not correlated with CRP or with clinical disease activity indexes (CDAI) [31]. Besides, in another study, Karmiris et al. compared serum levels of adipokines among UC, CD and healthy controls. Patients with UC and CD were divided into two groups: with disease activity and in remission [32]. Both studies showed no significant difference in the levels of the studied adipokines [31, 32]. Likewise, another study concluded that serum adiponectin did not show a significant difference between the activity and in remission CD groups [33]. Rodrigues et al. confirmed lower levels of serum adiponectin in active CD patients compared to healthy controls, but without significant difference compared to those in remission, and serum leptin was similar among the groups. This article expanded the analysis of adiponectin for mesenteric adipose tissue evaluation and verified lower expression in active CD as well, suggesting a deficient anti-inflammatory mechanism in this disease [21].

Corroborating the publications already cited, Karahman et al., confirmed that CD patients with CDAI <150 and  $\geq$ 150 did not show any significant difference regarding serum levels of adiponectin and leptin [34]. However, Trejo-Vasquez et al. assessed the serum level of adipokines and their association with endoscopic activity of the disease. They showed that serum leptin is decreased in CD patients compared to healthy controls, and even lower when comparing CD patients with disease activity to patients in remission. There was no significant difference in the serum concentration of other adipokines evaluated when comparing patients with or without disease activity [35].

### *Resistin*

Resistin is a central blocker of leptin, which induces satiety. Karmiris et al. did not identify any significant association of resistin serum levels and CRP or with Crohn's disease Activity index (CDAI) [31, 32]. Indeed, Trejo-Vasquez et al. found no significant differences in the

serum concentration assessed when comparing patients with or without disease activity [35]. On the other hand, Konrad et al. confirmed that serum resistin concentrations were significantly associated with elevated leukocytes, CRP and disease activity, suggesting that resistin is more sensitive to inflammatory processes than CRP [36]. These were consistent with the findings reported by Theocharidou et al., which showed significantly higher serum resistin levels in active CD compared to CD in remission [33].

### *Visfatin and PAI-1*

Visfatin and PAI-1 were cited only in a publication by Trejo-Vasquez et al., and they did not show a significant difference in the serum concentration of the evaluated adipokines when comparing CD patients with or without disease activity [35].

### *Ghrelin*

Ghrelin is a peptide hormone produced mainly by the epsilon cells of the stomach and pancreas and it is released in the bloodstream. Karmiris et al. and Trejo-Vasquez et al. did not find any significant difference in ghrelin serum concentration when comparing CD patients with or without disease activity [31, 35].

### *Omentin-1*

Omentin-1 is an adipocytokine of 313 amino acids, which is expressed in visceral omental tissue among others. This adipokine was studied by Yin et al., who demonstrated that its decrease in the serum can be considered an independent predictive marker of clinical disease activity for both CD and UC [37].

### *Interleukin-6*

This adipokine, unlike those mentioned above, was evaluated in human biopsies of intestinal mucosa. Christophi et al. concluded that IL-6, MCP-1 (CCL2), CCR2, CCL20, and IP-10 (CXCL10) were increased in active CD. TNF- $\alpha$ , IL-8 and IL-17A induced by NF-KB were elevated in active IBD [40]. Likewise, Leal et al. demonstrated that IL-6 responds to anti-TNF- $\alpha$  and is negatively regulated regardless of the therapeutic response. IL1B, S100A8, S100A9, CXCL2, CXCL6, S100A12, SLC64A14, FCG3B (CD-16b) and IL17A genes were upregulated in

patients with active CD. REG1A, IL-8, S100A8, S100A9, IL1B, MMP1, MMP3 are inflammation-dependent genes that are downregulated as mucosal lesion recovery occurs [41].

Boland et al. demonstrated the IL-6 expression separating by using biopsy topography. Thus, in the ileum, IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , IP-10, MMP-3 and S100-A8 were increased in active CD, while IL-6, IL-8, TNF- $\alpha$ , IP-10, MMP-3 and S100-A8 were increased in colonic active CD. Concerning the rectal mucosa, they identified higher levels of IL-8, TNF- $\alpha$ , IP-10, MMP3 and S100-A8 in active disease [42]. Yarur et al. analyzed blood samples and observed significantly lower levels of ICAM-1, IL-6 and TNF- $\alpha$  in patients with healed mucosa, i.e., in remission of the disease, as compared with the group with CD in activity. Reaffirming the hypothesis that the serum analysis may not reflect the inflammatory changes in the tissue [43]. However, a more recent article reported by Bourgonje et al. compared serum biomarkers with fecal calprotectin demonstrating that the increase of fecal calprotectin levels is associated to an increase of serum IFN- $\gamma$  and IL-6. It also had a significant correlation with serum SAA, PCR, IL-17A and TNF- $\beta$  [38].

One of the main limitations of the present review is the variety of the studies included in the analysis concerning the methods applied for the chemokine and adipokine analysis and the criteria used for the active CD definition. The different methods of cytokine quantification from distinct type of tissue samples besides the distinct inflammation criteria for analysis complicated the comparison among the studies. Most of the studies which addressed adipokines were based only on clinical criteria which may not always correlate with the presence of bowel lesions. Another limitation is the comparison between active CD patients with healthy controls but not with those in remission. For this reason, our SR is more descriptive than comparative, but it is still relevant since the data found here point towards chemokines and adipokines that should be explored in future large studies.

Moreover, an important limitation about chemokine and adipokine analysis in CD is that it has a vast role in inflammation. For example, the adipose tissue has been the major source

of the adipokine TNF- $\alpha$ , but other cells can also produce it as well, possessing a crucial pro-inflammatory function, and helping with resistance against infections and cancer [44]. Therefore, there is a wide range of scenarios that can influence TNF- $\alpha$  activity and make the association with some specific aspect of CD difficult. The IL-6 adipokine, as the TNF- $\alpha$ , is produced by macrophages, and only 30% of the circulating IL-6 is released by adipose tissue [44]. Even the ghrelin has the stomach and pancreas as a source, besides the adipose tissue. Thus, most of the adipokines can be influenced by diverse inflammatory processes besides the CD [45]. Similar to the adipokines, the chemokines can be influenced by other diseases and by acute inflammation [46].

### Conclusions

Despite the limitations and the small number of studies found in the literature that followed the criteria of this SR, some chemokines (CCL2, CCL20, CXCL1, CXCL3, CXCL8, CXCL10 and CXCL1) and adipokines (leptin, IL-6 and TNF- $\alpha$ ) have unveiled promising results that may enable us to distinguish active versus in remission forms of CD based on objective criteria of inflammation such as endoscopic, histologic or radiological criteria. Indeed, most chemokines and adipokines with significant levels were taken from peripheral blood, which is less invasive. Given the chronic character of CD, it is helpful to detect intestinal lesions before clinical symptoms become noticeable to avoid permanent bowel damage. With this in mind, less invasive biomarkers for CD, such as the ones we highlighted in this review, may prove to become effective tools for their management and follow-up.

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### Disclosure of conflict of interest

None.

### Abbreviations

CD, Crohn's disease; IBD, inflammatory bowel disease; MRI, entero magnetic resonance imaging; CRP, C-reactive protein; SR, systematic review; QCRI, Qatar Computing Research Institute; SMS, School of Medical Sciences; Unicamp, University of Campinas; CDAI, clinical disease activity index; PAI-1, plasminogen activator inhibitor-1; UC, ulcerative colitis; IL-6, interleukin 6; MCP-1, Monocyte chemoattractant protein-1; CCL2, C-C Motif Chemokine Ligand 2; CCR2, C-C chemokine receptor type 2; CCL20, C-C Motif Chemokine Ligand 20; IP-10, Interferon gamma-induced protein 10; CXCL10, C-X-C Motif Chemokine Ligand 10; TNF- $\alpha$ , Tumour necrosis factor  $\alpha$ ; IL-8, Interleukin 8; IL-17A, Interleukin-17A; NF- $\kappa$ B, factor nuclear kappa B; IL1 $\beta$ , Interleukin 1 Beta; S100A8, S100 Calcium Binding Protein A8; S100A9, S100 Calcium Binding Protein A9; CXCL2, C-X-C Motif Chemokine Ligand 2; CXCL6, C-X-C Motif Chemokine Ligand 6; S100A12, S100 calcium-binding protein A12; SLC64A14, solute carrier family 6 member 14; FCG3B, Fc Fragment of IgG Receptor IIIb; REG1A, Regenerating Family Member 1 Alpha; MMP1, matrix Metalloproteinase 1; MMP3, matrix Metalloproteinase 3; ICAM-1, Intercellular Adhesion Molecule 1; IFN- $\gamma$ , Interferon-gamma; SAA, Serum Amyloid A; TNF- $\beta$ , Tumor necrosis factor  $\beta$ ; CCL, C-C Motif Chemokine Ligand; CXCL, C-X-C Motif Chemokine Ligand; CX3CL, C-X3-C motif Chemokine Ligand; CCL25, C-C Motif Chemokine Ligand 25; CCR9, C-C chemokine receptor type 9; RT-PCR, Reverse Transcription Polymerase Chain Reaction; CCR6, C-C chemokine receptor type 6; mRNA, messenger ribonucleic acid; CCL19, C-C Motif Chemokine Ligand 19; CCL21, C-C Motif Chemokine Ligand 21; CCR7, C-C Motif Chemokine Receptor 7; ELISA, Enzyme-Linked Immunosorbent Assay; CCL3, C-C Motif Chemokine Ligand 3; CCL4, C-C Motif Chemokine Ligand 4; CCL11, C-C Motif Chemokine Ligand 11; CCL17, C-C Motif Chemokine

Ligand 17; CCL22, C-C Motif Chemokine Ligand 22; CCL26, C-C Motif Chemokine Ligand 26; CXCL1, C-X-C Motif Chemokine Ligand 1; CXCL8, C-X-C motif chemokine ligand 8.

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