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Effects of Environmental Contaminants in Inflammatory Bowel Disease: A Systematic Review

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"Water and air, the two essential fluids on which all life depends, have become global garbage cans"

Jacques Yves Cousteau

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Resumo Alargado

Introdução: A Doença Inflamatória Intestinal é caracterizada por uma inflamação crónica do trato gastrointestinal e possui duas formas importantes: Doença de Crohn e Colite Ulcerosa. Embora a fisiopatologia da doença seja praticamente desconhecida, as evidências recentes sugerem que esta patologia resulta da interação de diferentes fatores, nomeadamente, imunológicos, genéticos, o microbioma e o ambiente. O papel dos fatores ambientais na fisiopatologia desta doença é reforçado se considerarmos o rápido crescimento nas taxas de incidência e prevalência da Doença Inflamatória Intestinal em zonas onde a incidência era baixa, como é o caso da Ásia. No entanto, a bibliografia acerca do papel dos fatores ambientais na doença inflamatória intestinal é reduzida e não se encontra sistematizada.

Objetivos: Estudar o efeito dos contaminantes ambientais na doença inflamatória intestinal através de uma revisão sistemática da literatura.

Métodos: Para esta revisão sistemática foi utilizada a metodologia "Navigation Guide Method for Grading Human Evidence". A pesquisa foi realizada em três bases de dados (Scopus, Web of Science, PubMed/MEDLINE). Os critérios de inclusão utilizados foram: artigos de investigação com dados originais em humanos, doença inflamatória intestinal clinicamente diagnosticada, quantificação de contaminantes ambientais e estudo da associação entre contaminantes ambientais e doença inflamatória intestinal. Apenas artigos escritos português, inglês, francês ou espanhol foram incluídos.

Resultados: Foram incluídos nesta revisão sistemática 16 estudos. Destes, 13 estudaram a Doença Inflamatória Intestinal com referências para a Doença de Crohn e Colite Ulcerosa, 2 estudos incluem apenas pacientes com Doença de Crohn e 1 apenas pacientes com Colite Ulcerosa. Os contaminantes ambientais mais estudados foram dióxido de enxofre, dióxido de nitrogénio, monóxido de carbono, material particulado, ácido perfluorooctanóico, alumínio, ferro, zinco, cobre, dióxido de titânio, silicatos e endotoxinas. Identificamos também grupos de estudo, 4 relacionados com poluição do ar, 3 relacionados com a ingestão de água contaminada 3 relacionados com a dieta, 2 em que as concentrações séricas de contaminantes foram avaliadas, 1 estudou a exposição a pó doméstico, 2 tentaram caracterizar células de pacientes com Doença Inflamatória Intestinal e num estudo tentaram induzir colite. De uma forma geral, a qualidade do corpo de evidência é moderada a baixa, e o efeito dos contaminantes ambientais na Doença Inflamatória Intestinal não foi comprovado.

Discussão: Nesta revisão sistemática, identificamos várias classes diferentes de contaminantes ambientais, mas estudos que analisam a concentração real de contaminantes ambientais em matrizes biológicas são raros. Considerando os vários fatores de

confundimento que não foram tidos em consideração e a variabilidade entre estudos, torna-se difícil extrair conclusões sobre a influência destes contaminantes no início da Doença Inflamatória Intestinal ou em crises.

Conclusão: Com base num nível de qualidade moderado a baixo, a evidência reunida nesta revisão sistemática é insuficiente para avaliar o efeito dos contaminantes ambientais na doença inflamatória intestinal, pelo que novos estudos epidemiológicos necessitam ser realizados.

Palavras chave: Doença Inflamatória Intestinal; Doença de Crohn; Colite ulcerosa; fatores de risco; contaminantes ambientais.

Abstract

Introduction: The Inflammatory Bowel Disease is characterized by a chronic inflammation of the gastrointestinal tract and it has two important forms: Crohn's Disease and Ulcerative Colitis. Although the pathophysiology of the disease is mostly unknown, an interaction between immune system, genetics, microbiome and environmental factors seem to be responsible for the disease onset. The role of environmental factors in the pathophysiology of this disease is reinforced if we consider the rapid growth in incidence and prevalence rates of Inflammatory Bowel Disease in areas where the incidence was low as for example Asia. However, the literature on the role of environmental factors in Inflammatory Bowel Disease is reduced and not systematized.

Objectives: To study the effect of environmental contaminants on Inflammatory Bowel Disease through a systematic review of the literature.

Methods: For this systematic review the methodology "Navigation Guide Method for Grading Human Evidence" was used. The research was carried out in three databases (Scopus, Web of Science, PubMed / MEDLINE). The inclusion criteria used were: research articles with original human data, clinically diagnosed Inflammatory Bowel Disease, quantification of environmental contaminants and study of the association between environmental contaminants and Inflammatory Bowel Disease. Only written articles Portuguese, English, French or Spanish were included.

Results: Sixteen studies were included in this systematic review. Of these, 13 studied Inflammatory Bowel Disease in general, 2 studies included only patients with Crohn's Disease and 1 study included only patients with Ulcerative Colitis. The most studied environmental contaminants were sulphur dioxide, nitrogen dioxide, carbon monoxide, particulate matter, perfluorooctanoic acid, aluminum, iron, zinc, copper, titanium dioxide, silicates and endotoxins. We also identified different categories, 4 studies on the effects of air pollution, 3 on the intake of contaminated water, 3 on the effects of diet. Additionally, 2 studies evaluated the serum concentrations of contaminants, 1 studied the impact of exposure to house dust, 2 tried to characterize cells of patients with Inflammatory Bowel Disease and in one study colitis was induced. Overall, the quality of evidence is moderate to low, and there is inadequate evidence on the effect of environmental contaminants on Inflammatory Bowel Disease.

Discussion: In this systematic review, we have identified different classes of environmental contaminants, but studies analyzing the concentration of environmental contaminants in biological matrices are rare. Given the different confounding factors that were not taken into

account and the variability between studies it is difficult to draw conclusions on the real role of environmental contaminants on the onset of Inflammatory Bowel Disease or in flares.

Conclusion: Based on a moderate to low level of quality, the available evidence gathered in this systematic review is insufficient to access the effects of environmental contaminants Inflammatory Bowel Disease. New epidemiological studies are necessary.

Keywords: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Risk factors; Environmental contaminants.

Index

List of Figures

List of Tables

Acronyms

CD – Crohn's Disease

EPICN – European Prospective Investigation into Cancer and Nutrition

IBD – Inflammatory Bowel Disease

NHS – Nurses Health Study

PM2.5 – Particulate matter with an aerodynamic diameter of <2,5 μm

PM10 – Particulate matter with an aerodynamic diameter of <10 μm

PFOA - Perfluorooctanoic Acid

THIN – The Health Improvement Network

UC - Ulcerative Colitis

Introduction

The Inflammatory Bowel Disease (IBD) is characterized by a chronic inflammation of the gastrointestinal tract and it has two important forms: Crohn's Disease and Ulcerative Colitis. Both forms of presentation differ on their characteristics (1).

Crohn's Disease (CD) can affect all parts of the gastrointestinal tract, from mouth to anus. It has a discontinued presentation, with areas where the disease is present interleaved with healthy areas, however, the most affected area in Crohn's Disease is the ileum. Crohn's Disease can affect all the bowel wall allowing the creation of fistulas to the urinary tract or vagina leading to fecaluria or pneumaturia, that is a distinctive mark in relation to Ulcerative Colitis (2).

On the other hand, in Ulcerative Colitis (UC) only the colon and rectum are affected. Ulcerative colitis has a continuous presentation without interleaved healthy areas and only affects the inner layers of the colon and rectum areas (2).

Both forms have similar symptoms like diarrhea, abdominal pain, rectal bleeding and sensation of incomplete evacuation. General symptoms like fever, loss of appetite, weight loss, fatigue and in women loss of normal menstrual cycle can also be present (1,2).

Extra-intestinal presentations of the disease can also occur, normally associated with arthropathy, uveitis, erythema nodosum, metabolic bone diseases, kidney stones, osteoporosis amongst others (2).

Diagnosis of IBD is mostly performed by endoscopy with biopsy being the only method that allows to differentiate Crohn's Disease from Ulcerative Colitis (2).

Traditionally, Inflammatory Bowel Disease is associated with developed countries, mostly in Northern Europe or North America, with the highest incidence and prevalence being found for Ulcerative Colitis (3).

Epidemiological studies conducted across countries showed a sharp increase in rates of incidence and prevalence by the turn of the century in countries from Northern Europe. In Denmark, for example, between 1962-1987 the incidence by 100 000 was 4.1 for Crohn's Disease and 8.1 for Ulcerative Colitis. In the beginning of the XXI century (between 2003-2005) these numbers increased to 8.6 for Crohn's Disease and 13.4 for Ulcerative Colitis (4).

Presently, with the western countries benefiting of a stabilization on incidence, the biggest rise in Inflammatory Bowel Disease is being observed in areas for which IBD expression was not important in the past (5). In fact, Asia region is witnessing an increasing incidence of IBD over the recent years (6). In South Korea, for example, between 1986-1990, an incidence by 100 000 people of 0.34 and 0.05 for Ulcerative Colitis and Crohn's Disease was reported, respectively. More recently, studies from 2006-2012 show a prevalence of 3.20 and 4.60 for Crohn's Disease and 3.20 for Ulcerative Colitis, respectively (6). This sharp increase in Asian countries, contrasts with the trends observed in western societies, where the prevalence is still higher but the increasing trend is not so notorious (4,5).

Pathophysiology

Inflammatory Bowel Disease pathogenesis is mostly unknown. Although it results from an immunological process, an interaction between different factors seem to be the cause for the intestinal immunological alterations. Besides immunological factors, genetics, gut microbiome and environment are considered to play a key role in this disease (7).

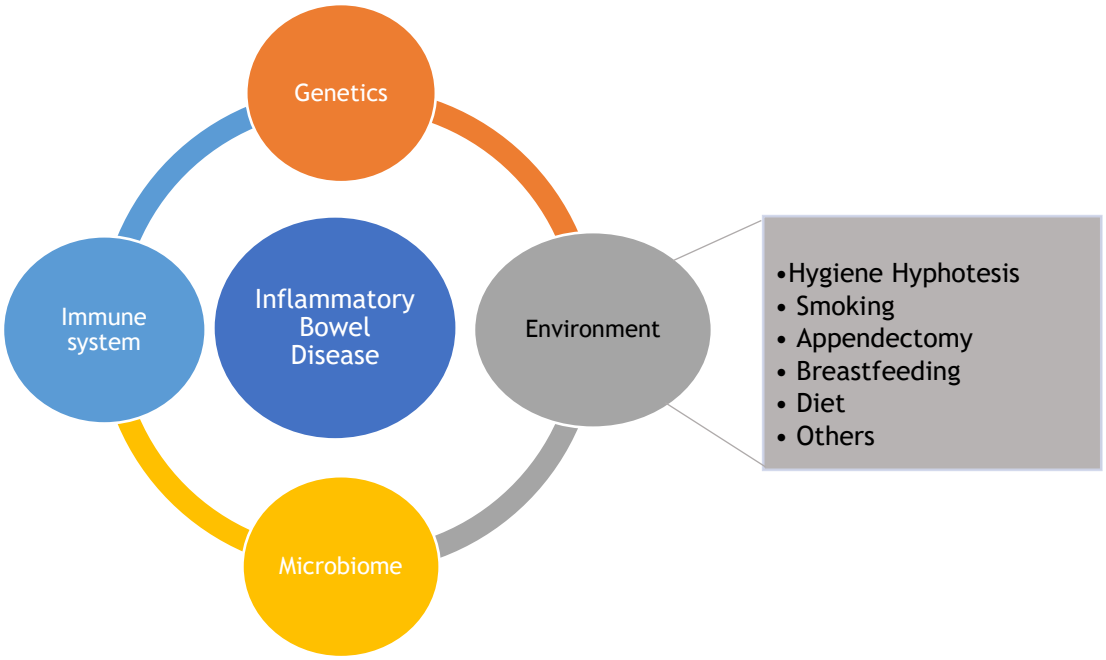

Figure 1 - Important factors in Inflammatory Bowel Disease.

Genetics

The importance of genetics in Inflammatory Bowel Disease arose from familial aggregation studies and from studies with twins. These studies were pivotal to show that Crohn's Disease and Ulcerative Colitis have a hereditary component. In fact, twin studies showed that in monozygotic twins a concordance rate of 20%-50% was present when assessing Crohn's

Disease, in Ulcerative Colitis concordance rate downs to 16%. In dizygotic twins, concordance rates are lower for both the diseases (7).

The results obtained from familial aggregation studies lead the scientific community to investigate possible genes involved in Inflammatory Bowel Disease. Some genes were already described as possible influencers in Crohn's Disease onset, namely: Nucleotide-binding oligomerization domain containing 2 gene, IRGM, ATG16L1, IL23R and PTPN2 and for Ulcerative Colitis genes like RNF186, IL23R, CARD9 (8,9).

Microbiome

The intestinal microbiome is important to maintain normal physiological function, and its deregulation can be envisaged as a factor for the development of alterations in the immune system (10). Microbiome does not directly contact with the cells responsible for immunity that are present on the Gut Associated Lymphoid Tissue or in the Lamina Propria. The first barrier is the secretion of antimicrobial peptides and mucus (heavily glycosylated mucins) secreted by intestinal epithelial cells, more specifically the Goblet Cells and also that imprisons the bacteria on its surface not allowing direct contact with any of the intestinal epithelial cells, whether they are - entero-absorptive enterocytes, entero-endocrine cells, goblet cells, or Paneth cells. Secondarily, intestinal epithelial cells have tight junctions that do not allow contact between the intestinal lumen and the lamina propria. Thus, for the cross-talk between microbioma and immune cells to occur, this must be performed through and with the "collaboration" of this monolayer of intestinal epithelial cells (10,11) .

Studies show that in patients with Inflammatory Bowel Disease a dysbiosis is present, with a higher presence of bacteria like adherent-invasive *Escherichia coli*, *Fusobacterium* and *Enterobacteriaceae* (12), and a lower presence of commonly present bacteria like *Firmicutes* and *Bacteroidetes* (10).

Environmental factors

The importance of environmental factors for the development of Inflammatory Bowel Disease is a hot topic when assessing the pathogenesis of the disease. The interaction between environmental exposures and individuals has been suggested to be an important factor for the disease onset. This possible association is corroborated by the sharp increase of Inflammatory Bowel Disease in regions traditionally not affected (e.g. Asian countries) and by the fact that immigrants from regions with low incidence and prevalence exhibit a higher risk of developing Inflammatory Bowel Disease when the migration occurs to countries with higher incidence and prevalence (13,14). Despite such evidences, there is limited information available on what concerns exposure to environmental contaminants and IBD. Furthermore, the information available is limited to specific environmental risks. In fact, the available literature on environmental factors associated with IBD is mostly focused in the hygiene hypothesis, smoking, appendectomy, breastfeeding and diet (15,16) and studies on the association of IBD

with specific classes of contaminants like metals or endocrine disruptors are very limited (17,18).

The Hygiene Hypothesis suggests that the increased incidence of immunological diseases is associated with reduced exposure, during childhood, to pathogens. Exposure to *Helicobacter pylori* is reported to protect against Inflammatory Bowel Disease since these bacteria increase the expression of genes that regulate the function of T-regulatory cells (15). Living in a rural area and the interaction with pets and with farm animals in young ages are also considered to be protective factors since they presented a reduction in the incidence of Inflammatory Bowel Disease (15,19). However, the role of hygiene hypothesis in Inflammatory Bowel Disease still lacks robust confirmation and more studies are warranted (7).

Over the years, smoking has been pointed out as a risk factor for the development of Crohn's disease and as a protective factor in the development of Ulcerative Colitis. However, despite being the most studied risk factor, the mechanisms by which it acts and that lead to the onset of IBD are poorly understood (20). In the case of Crohn's disease, smoking is indicated as an independent risk factor and is associated with the appearance of a more severe and more refractory Crohn's Disease. On the contrary, smoking cessation is associated with fewer exacerbations and allows the use of lower amounts of corticosteroids and immunosuppressants (20). In Ulcerative Colitis, tobacco is protective, and it is associated with a less severe course (20). Some studies suggest that in Ulcerative Colitis, smoking is associated with lower hospitalization rates, reduced need for corticosteroids, as well as fewer exacerbations and fewer colectomies (21). However, these possible smoking benefits are not consensual, and a meta-analysis published in 2016 concluded that in smokers with Ulcerative Colitis, tobacco did not reduce neither the number of exacerbations nor the number of colectomies when compared with ex-smokers or smokers (22).

Appendectomy is also a factor highly studied when trying to understand the pathogenesis of Inflammatory Bowel Disease. Scientific knowledge seems to postulate a negative association between appendectomy and Ulcerative Colitis mostly when children are under 10 years old. However, the reasons by which appendectomy protects against Ulcerative Colitis are unknown. When analyzing the relation with Crohn's Disease, there is no clear association with studies presenting both a positive and a negative association (15,19).

Although breastfeeding and diet have been studied as risk factors for Inflammatory Bowel Disease, they have different effects on its pathogenesis (15). Association with breastfeeding and increase in Inflammatory Bowel Disease's incidence has not been reported (13,16). Diet, on the other hand, may have an effect on IBD when considering the consumption of sugar, fibers, fruits and vegetables and fats (15,23).

Overall, the research on the environmental risk factors for Inflammatory Bowel Disease is still in its infancy. In fact, the majority of the available studies failed to address environmental

risk factors derived from industrial processes and commonly found in western societies.

This lack of studies is of concern if we consider the increasing incidence of Inflammatory Bowel Disease in regions suffering from a rapidly industrialization and from the adoption of westernized cultural habits (5,7). As such, studies dealing with for example exposure to air pollution and exposure to chemical and biological agents such as metals and toxins are of utmost importance. Considering that there are some studies already available on this topic, the aim of this thesis is to gather the available information in order to evaluate if exposure to environmental contaminants is or not associated with Inflammatory Bowel Disease. In order to achieve this goal a systematic review of the literature was performed.

Methodology

Systematic Review

This systematic review was elaborated following the "Navigation Guide Method for Grading Human Evidence" (24). The steps used in this systematic review are depicted in figure 2.

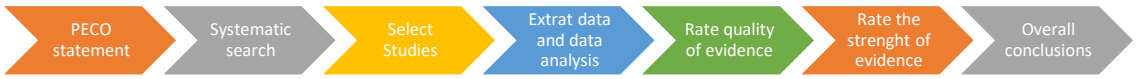

Figure 2 - Navigation Guide protocol.

PECO Statement:

PECO is a tool used to answer a question of health impact. The question defined for this systematic review was: "Is there a relation between Inflammatory Bowel Disease and Environmental Contaminants exposure?". Each letter in PECO means a word: P for population, E for exposure, C for Comparison and O for Outcomes.

- **Population:** Patients with Inflammatory Bowel Disease and Controls
  Population included in this study didn't have an age range. All ages were included.
- **Exposure:** Exposure to environmental contaminants
  Exposure was defined by a quantification of an environmental contaminant in any biological or environmental sample.
- **Comparison:** Between humans with Inflammatory Bowel Disease and healthy individuals.
- **Outcomes:** Inflammatory Bowel Disease onset or flares.

Systematic search

The search was conducted in three databases: SCOPUS, Web of Science and PubMed/MEDLINE. The keywords used were: "Inflammatory bowel disease" OR "IBD" OR "Crohn's disease" OR "ulcerative colitis" AND "risk factors" OR "Environmental factors" OR "Environment" OR "Contaminants" OR "Metal" OR "pollutants" OR "pollution". Studies in languages other than English, French, Spanish and Portuguese were excluded.

Table 1 - Parameters used in the systematic search and respective results.

Database	Date	Search Terms	Search Field Type	N° of Results
Scopus	10/12/2017	"Inflammatory bowel disease" OR "IBD" OR "Crohn's disease" OR "ulcerative colitis" AND "risk factors" OR "Environmental factors" OR "Environment" OR "Contaminants" OR "Metal" OR "pollutants" OR "pollution"	Article title, Abstract, Keywords	12,862
Web of Science	10/12/2017	"Inflammatory bowel disease" OR "IBD" OR "Crohn's disease" OR "ulcerative colitis" AND "risk factors" OR "Environmental factors" OR "Environment" OR "Contaminants" OR "Metal" OR "pollutants" OR "pollution"	Topic	6,270
PubMed/Medline	10/12/2017	"Inflammatory bowel disease" OR "IBD" OR "Crohn's disease" OR "ulcerative colitis" AND "risk factors" OR "Environmental factors" OR "Environment" OR "Contaminants" OR "Metal" OR "pollutants" OR "pollution"	Title/Abstract	4,007

Study selection

The studies were select independently by two reviewers. All the studies identified in the search were extracted and all duplicates were removed by the software EndNote X8. Then, all the remaining articles passed through a tittle screening, where all the articles with a relevant title were selected. The next phase was screening all the abstracts by both reviewers. The articles considered relevant after abstract screening passed to a full reading phase where they were confronted with inclusion/exclusion criteria. Articles for which the abstract reading was inconclusive were also included in this full text reading phase. In cases of disagreement regarding the eligibility of a study, a third reviewer intervened.

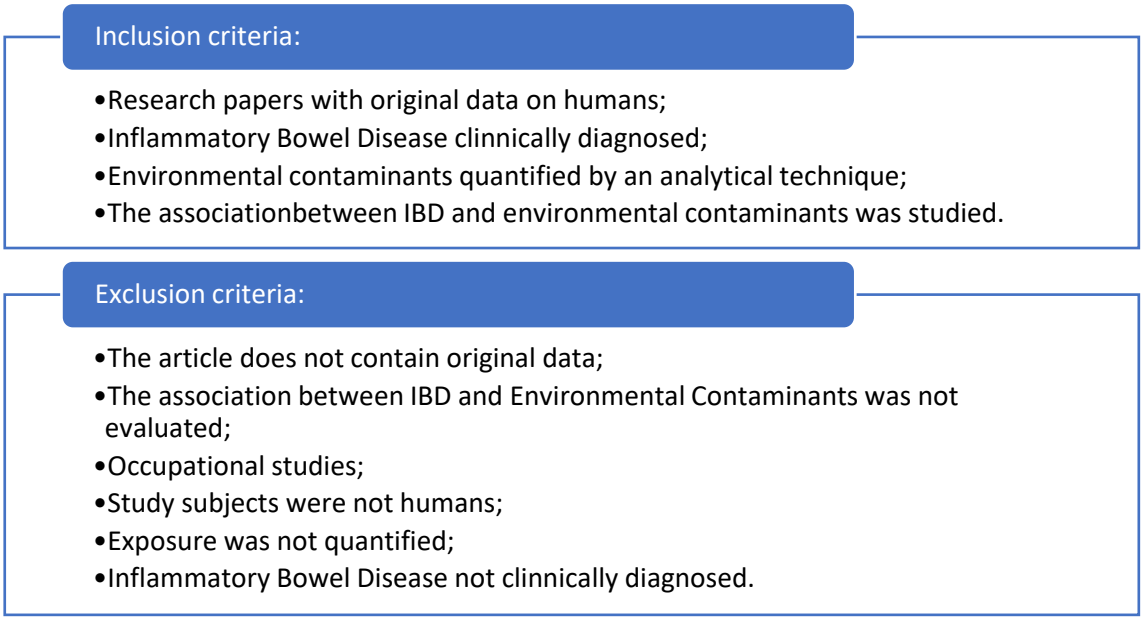

Figure 3 - Inclusion and exclusion criteria.

Data extraction

Of the selected studies, the following data was extracted: year, country, participant characteristics, study design, biological matrices analyzed, environmental matrices analyzed, age (mean and standard deviation), sample size (cases and controls separately, whenever possible), contaminants, IBD Classification (Crohn's Disease or Ulcerative Colitis), flares, odds ratio, confidence interval.

Quality and strength of evidence

In the "Navigation Guide Systematic Review Methodology" the quality of the evidence is rated for each of study using specific domains. After analysing all the studies included in the systematic review, the global assessment for the quality of evidence is performed taken into account all the factors that can decrease or increase the quality of evidence. In the end, the strength of evidence allow us to conclude if we have or not a positive effect of an exposure and an outcome (24).

Risk of Bias

Bias is a systematic error that can under or overestimate the importance of a result. To evaluate the risk of bias in this systematic review we also used the protocol from the "Navigation Guide Systematic Review Methodology". The protocol is presented in figure 4 (24).

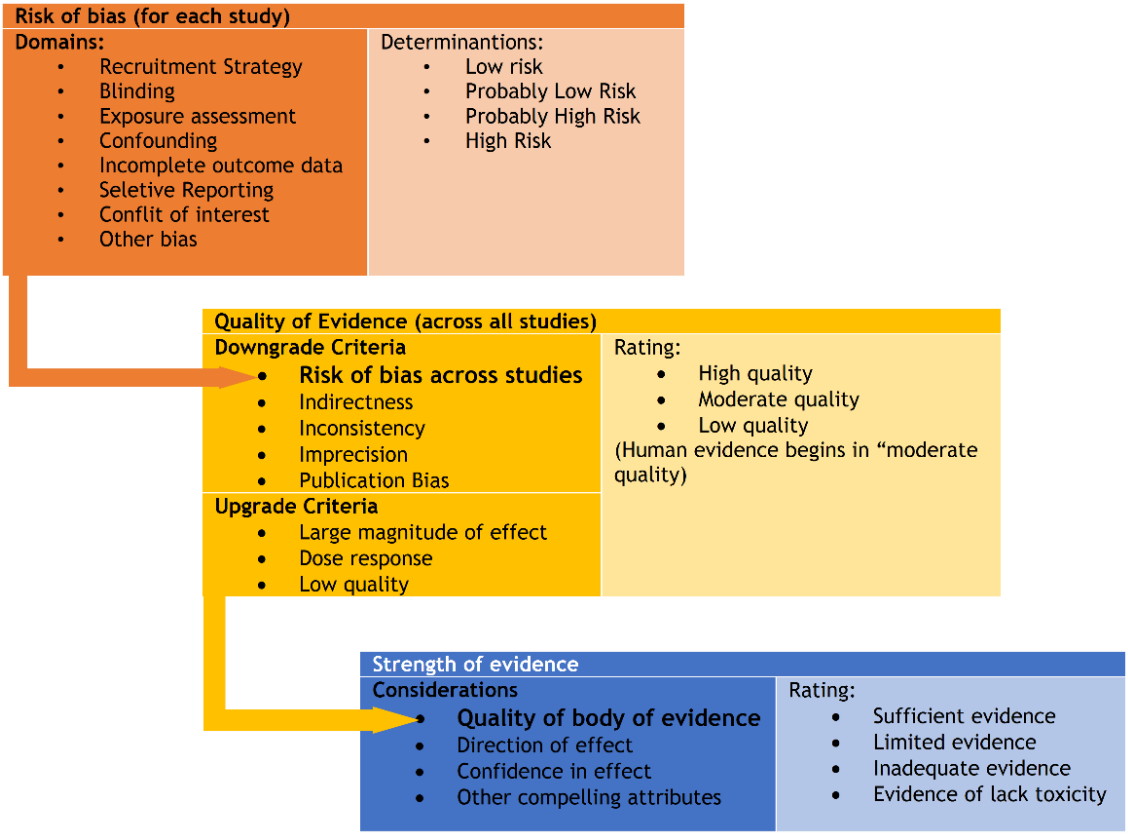

Figure 4 - Diagram representing the "Navigation Guide Systematic Review Methodology" protocol used to classify the quality and strength of evidence in humans. Adapted from Johnson et al. (25).

Each of the eight domains that evaluate the risk of bias were classified into “low risk”, “probably low risk”, “probably high risk” and “high risk” according to Lam et al. (25).

- **Recruitment Strategy:** Participant selection protects against selection bias;
- **Blinding:** Knowledge of exposure is prevented when assessing outcome;
- **Exposure Assessment:** Risk of exposure misclassification is minimized through validated methods;
- **Confounding:** Important potential confounders were appropriately accounted for;
- **Incomplete Outcome Data:** Any missing outcome data is not likely to introduce bias;
- **Selective Reporting:** All outcomes specified in methods have been reported;
- **Conflict of Interest:** Study free of support from individual or entity having financial interest in outcome of the study.

The quality of evidence was assessed across all studies considered in this systematic review. Since we are working with human evidence, the Navigation Guide Protocol proposes to start with “Moderate evidence”. Then, we can rate down with factors that decrease quality (-1 or -2) and we rate up with factors that increase quality (+1 or +2) (24,25).

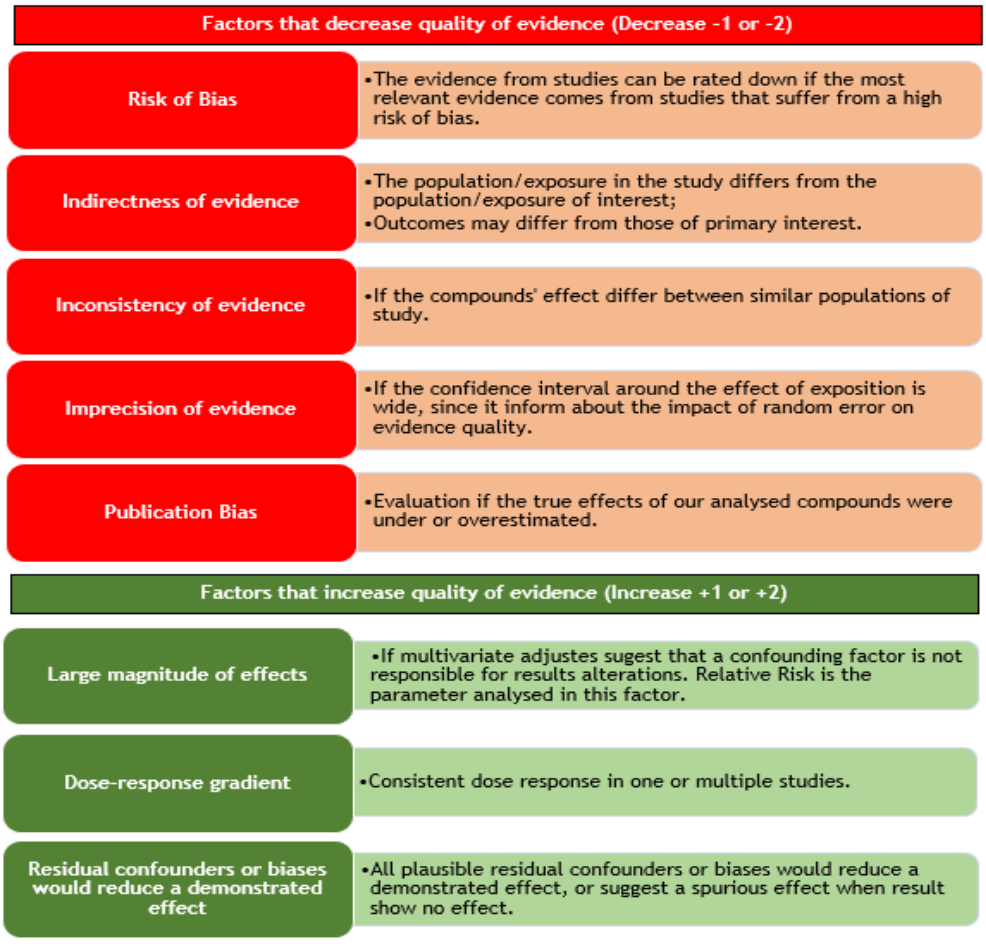

Figure 5 - Factors to consider when assessing the quality of evidence. Adapted from Lam et al (25).

To assess the strength of evidence four parameters need to be considered: quality of the body of evidence, direction of effect, confidence in effect and other compelling attributes. This will lead us to the rating of strength of evidence as: “Sufficient evidence of toxicity”, “Limited evidence of toxicity”, “Inadequate evidence of toxicity” or “Lack evidence of toxicity” (26).

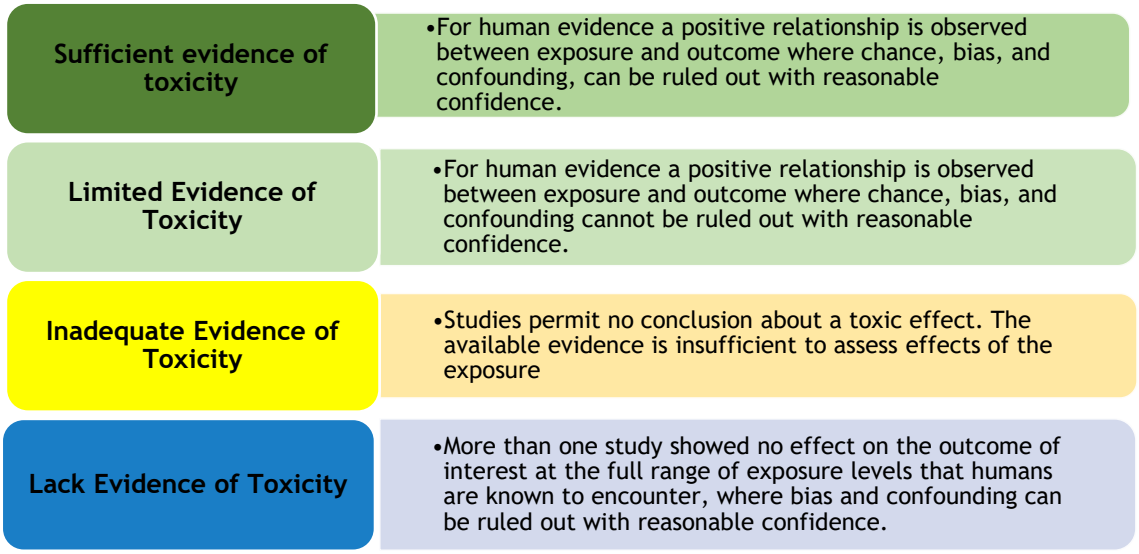

Figure 6 - Strength of evidence classification. Adapted from University of California (26).

Results

Study Selection

The systematic search resulted in 23139 articles, of those 7113 were duplicates and were immediately removed. From the 16026 that remained, 58 were potentially eligible after title and abstract screening.

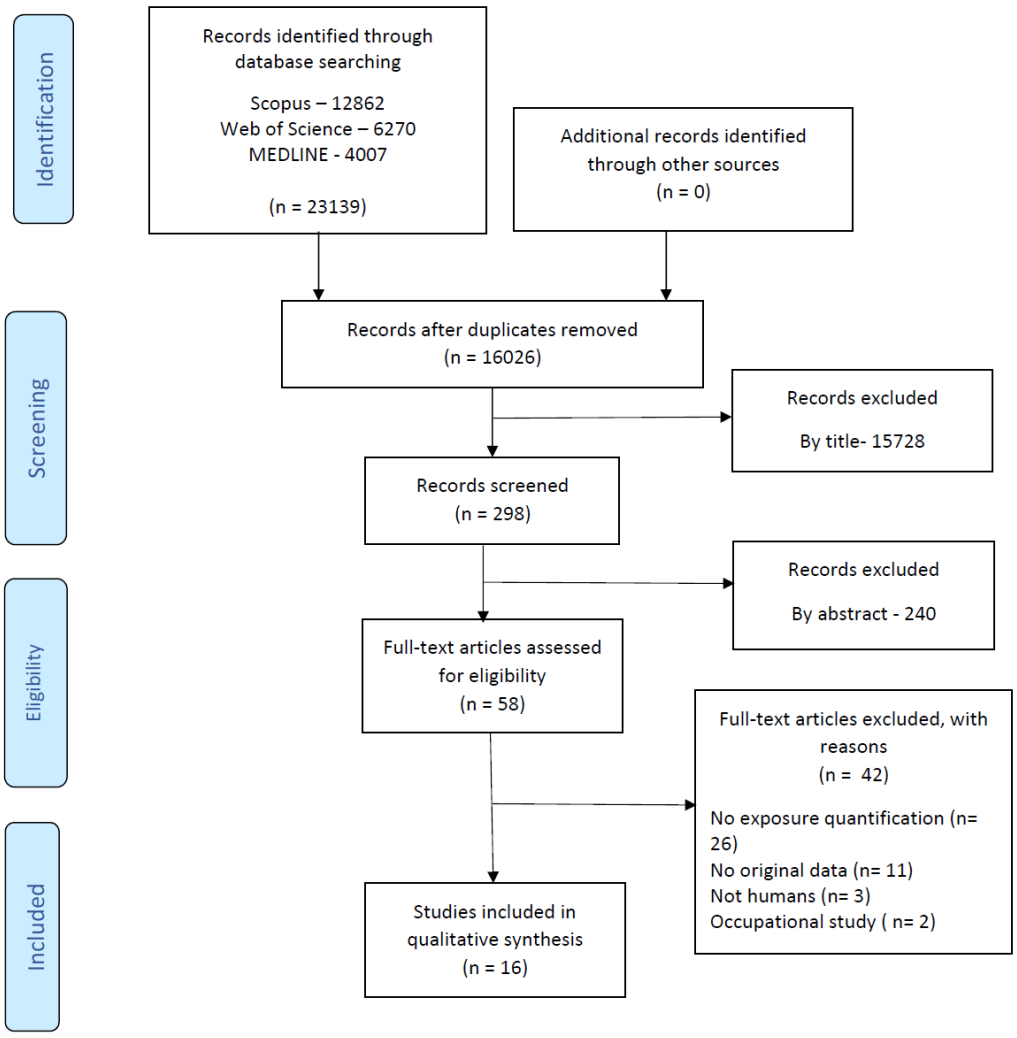

Figure 7 - Diagram for study selection.

Study Characteristics

From the sixteen studies included in the analysis, 13 studies were related with Inflammatory Bowel Disease (both Crohn's Disease and Ulcerative Colitis)(27–39), 2 analysed the influence of contaminants only in Crohn's Disease (40,41) and 1 only in Ulcerative Colitis (18).

The studies included in this systematic review, analysed different contaminants and also different exposure pathways. Inhalation of contaminated air and ingestion either of contaminated drinking water or diet were the most frequent exposure pathways addressed. The most frequently addressed contaminants were: carbon monoxide, nitrous oxide, sulphur dioxide and suspended particulate matter of size less than 2.5 μm (PM2.5), ozone, iron, manganese, 1,2-dichloroethane, aluminium, ammonium, nitrite, all trihalomethanes collectively and all tetra- and trichloroethanes collectively, zinc, titanium dioxide, perfluorooctanoic acid and endotoxins.

All the studies related to ambient air exposure used data from patients' databases as their source to find patients with Crohn's Disease and Ulcerative Colitis. Ananthakrishnan et al. (2011) used data from Wisconsin Hospital Association (37), Kaplan et al. (2010) collected data from The Health Improvement Network Database (39). Nejad et al. (2016) studied the rate of flare and average duration of hospitalization in patients with Inflammatory Bowel Disease using data from Imam Hospital (38) and the study population from Opstelten et al. (2016) belong to the European Prospective Investigation into Cancer and Nutrition (36).

Ananthakrishnan et al. (2011) studied volatile organic compounds, carbon monoxide, nitrous oxide, sulphur dioxide and suspended particulate matter of size less than 2.5 μm. The air pollution data was obtained for 2002 from US Environmental Protection Agency. Means were 1578.9 tons/square miles for carbon monoxide, 10.7 tons/square miles for nitrous oxide, 7.9 tons/square mile for sulphur dioxide, 12.1 tons/square mile for volatile organic chemicals and 2.0 tons/square mile for particulate matter <2.5 μm. The authors found and increase in Inflammatory Bowel Disease hospitalization rate (incidence rate ratio 1.40; 95% confidence interval 1.31 – 1.50; $p< 0.001$) and the exposure to these compounds. Similar findings were obtained for Ulcerative Colitis (incidence rate ratio 1.48; 95% confidence interval 1.27 – 1.73; $p=0.03$) and Crohn's Disease (incidence rate ratio 1.39; 95% confidence interval 1.26 – 1.52; $p=0.005$) (37).

Kaplan et al. (2010) collected information related to particulate matter with an aerodynamic diameter of less than 10 μm, sulphur dioxide and nitrogen dioxide from the National Environmental Technology Centre and Department of Environment, Food and Rural Affairs from the United Kingdom. The results of the study suggested that nitrogen dioxide, sulphur dioxide and PM10 were not associated with the risk of developing Inflammatory Bowel Disease (39).

Nejad et al. (2016) included data collected between May 2012 and February 2013 from the Ahvaz aerology stations and Ahvaz Jundishapur University for carbon monoxide, sulphur dioxide, ozone and nitrogen dioxide. The authors concluded that there was a positive association, not statistically significant between carbon monoxide concentration and the number and duration of Ulcerative Colitis flares (p=0.135 and p=0.08 respectively) and any other compound (sulphur dioxide, ozone and nitrogen dioxide) didn't seem to influence Inflammatory Bowel Disease flares (38).

Opstelten et al. (2016) obtained air pollution data from the European Study of Cohorts for Air Pollution Effects like PM10, PM2.5, nitrogen oxides and nitrogen dioxide. The authors only found a positive association between air pollution exposure and Crohn's Disease for nitrogen dioxide (odds ratio 1.13; 95% confidence interval 0.59 – 2.16), any other association for Crohn's Disease or Ulcerative Colitis were found (36).

The effects of exposure to environmental contaminants trough the ingestion of contaminated water was evaluated in three studies (17,18,31). Aamodt et al. (2008) used patients with suspected Inflammatory Bowel Disease from the southeaster region of Norway. These patients were suspected to have Inflammatory Bowel Disease and the diagnostics was confirmed after a 5-year follow-up. Exposure assessment information was obtained from the Norwegian Institute of Public Health since the information of purified water needs to be reported to this institute. Information of iron, aluminium, acidity, colour, turbidity and coliform bacteria were evaluated. A positive association for Crohn's Disease and iron (rate ratio 1.22; 95% confidence interval 1 – 1.48; p = 0,0049) and Ulcerative Colitis with iron (rate ratio 1.17; 95% confidence interval 1.03 – 1.33; p = 0.0018) was found (17).

Lehtien et al. (2016) retrieved their data from the Finish Social Insurance Institution including all patients with diagnosis of Inflammatory Bowel Disease and aged between 0 and 14 between 1987 to 2003. When assessing the contaminants in water they considered iron, manganese, 1,2-dichloroethane, aluminium, ammonium, nitrite, all trihalomethanes collectively and all tetra- and trichloroethanes collectively. In this study no association between metals and Inflammatory Bowel Disease was found (31).

Steenland et al. (2013) studied a community from Mid-Ohio Valley including people living or working in any of six Perfluorooctanoic Acid-contaminated water districts and that participated in the C8 Health Project baseline survey in 2005-2006. The community cohort comprised 28541 residents that never worked in DuPont plant and the work cohort 3713 participants that have a past or present working relation with the DuPont plant, a residential and occupational exposure was estimated. Exposure assessment was conducted by estimating the annual mean serum Perfluorooctanoic Acid (PFOA) levels during the follow-up. Analysis were conducted by quartile of cumulative exposure. The authors found a positive increase in the incidence of ulcerative colitis when participants were exposed to PFOA. All quartiles comparison (Q2 vs Q1, Q3 vs Q1, Q4 vs Q1) shows a rate ratio 1.76 (95% confidence interval

1.04 – 2.99), 2.63 (95% confidence interval 1.56 – 4.43) and 2.86 (95% confidence interval 1.65 – 4.96) and a p-value of less than 0.0001 (18).

Diet is considered to play a pathogenically role in Inflammatory Bowel Disease. In this systematic review, three papers dealing with the exposure to environmental contaminants trhough diet were included (35,40,41). Ananthakrishnan et al. (2015) studied the zinc intake and risk of Crohn's Disease and Ulcerative Colitis. They selected their study population from women nurses participating in Nurses' Health Study (NHS) I and II. The Nurses' Health Study I was established in 1976 with nurses age between 30 and 55 years and in the Nurses' Health Study II the ages were between 25 and 42 years. These women were followed for 26 years. All women with a previous diagnosis of Inflammatory Bowel Disease, cancer except for non-melanoma skin cancer and that had died were excluded. Final cohort for study was constituted by 269 cases for Crohn's Disease and 338 cases for Ulcerative Colitis. To assess zinc intake, semi-quantitative food frequencies questionnaires were used every 4 years. To assess the nutrient intake food tables from the Department of Agriculture were provided. Consumption of multivitamin complex and zinc supplements were also assessed and brand, duration and frequency were asked. A stratification by daily intake of zinc in five groups (quintile 1: 3.0 – 9.9 mg/day; quintile 2: 10.0 – 11.2 mg/day; quintile 3: 11.0- 12.8 mg/day; quintile 4: 12.9 – 18.5 mg/day; quintile 5: 16.0 – 321.3 mg/day) was performed. The multivariate hazard ratios for Crohn's Disease were 0.92 (95% confidence interval 0.65 - 1.29) for second quintile, 0.60 (95% confidence interval 0.40 – 0.89) for the third quintile, 0.57 (95% confidence interval 0.38 – 0.86) for the fourth quintile and 0.74 (95% confidence interval 0.50 – 1.10) for the fifth quintile (35).

Lomer et al. (2001) studied the efficacy and tolerability of a low microparticle diet in Crohn's disease patients. The two microparticles important in this study were titanium dioxide and aluminosilicates that are naturally occurring substances but they are also added to food. Authors selected twenty-six patients with Crohn's Disease and ileal involvement. Twenty patients initiated the study since 6 were excluded due to pregnancy, refusal of dietary changes and insufficiently active disease, then these twenty patients were randomly selected to receive a diet low in microparticles or a control diet. Eighteen patients complete the study. To assess the difference between a normal diet and a low microparticle diet, the group receiving this diet received a list of food to be avoided, and fresh fruit and vegetables needed to be peeled and washed. Toothpaste without titanium dioxide was recommended and water that was filtered and bottled was supplied for all drinks, cooking, teeth cleaning and washing of fruit/vegetables. The control group received advices like the trial group, but foods containing microparticles were not discouraged. Both the case and control group were followed for 4 months. Authors concluded that a low microparticle diet was associated with a decrease in the Crohn's Disease activity index from entry to month 4, and seven where in remission (Crohn's Disease activity index <150) (41).

Lomer et al. (2004) recruited patients with Crohn's Disease from September 1999 to August 2000 by invitation letter from United Kingdom hospitals. Healthy controls (i.e. no known history of gastrointestinal disease) were randomly selected. Ninety-one cases and 91 controls were selected for this study. For dietary assessment researchers used a 7 days food diary to collect data on food intake. Pharmaceutical, dietary supplements and toothpaste usage was also assessed. To evaluate intake of titanium dioxide and mixed silicates from diet, pharmaceuticals, dietary supplements and toothpaste were obtained by label information, manufactures and analytical technique for titanium dioxide. No significant differences in these two inorganic particles intake was observed between cases and controls. Given such results the authors concluded that if these microparticles could be associated with Crohn's Disease the present study didn't find the excess intake as the problem (40).

As previously mentioned, only two studies evaluated the levels of environmental contaminants in patients' samples. Ringstad et al. (1993) studied the serum from 47 patients with Crohn's Disease, 117 patients with ulcerative colitis and 123 healthy controls belonging to a multicentre survey under the auspices of the Northern Norway Gastrointestinal Society. Serum was collected when patients had the first attack of the disease, which means that patients were not yet taking medications. Copper, zinc and selenium were analysed. Copper and zinc concentrations were analysed by atomic absorption spectroscopy and selenium concentrations by electrothermal atomic absorption spectroscopy after dilution with a nickel matrix modifier. A significant risk of having Crohn's Disease with decreasing levels of selenium or increasing levels of copper was found ($p<0.001$). When considering Ulcerative Colitis, men and women presented a significantly higher serum copper and serum zinc concentration than controls (28).

Thilo-Körner et al. (2000) analysed serum concentration from 428 patients that were registered in the internal medicine department from January 1995 and July 1997. Analysis for aluminium, cadmium, cooper, ion, lead, selenium and zinc were conducted. The results disclose that in a selected group of patients with Crohn's Disease, 40.9 % of them showed zinc deficiency. Furthermore, a patient with Crohn's Disease exhibited a serum aluminium's concentration of 182 µg/L (29).

Boneberger et al. (2011) evaluated the possible association between endotoxin levels in house dust and juvenile Inflammatory Bowel Disease. To select patients for this study, the authors contacted 331 patients that participated in a multi-center case-control study on animal contact and Inflammatory Bowel Disease. Since only cases and controls with complete questionnaire data and dust analyses were included, study end up with 176 patients, 85 cases with Inflammatory Bowel Disease and 91 controls without Inflammatory Bowel Disease. To assess exposure, a questionnaire was applied and a dust sample was collected. The questionnaire asked about sociodemographic characteristics and patient's early and present environmental exposure. The dust sample was collected from the residential vacuum cleaner

in the living room. Endotoxin levels were assessed by Limulus Amoebocyte Lysate assay. The authors concluded that there is an inverse association between Inflammatory Bowel Disease and endotoxin exposure, although it is not statistically significant (adjusted odds ratio 0.70; 95% confidence interval 0.46 – 1.04) (27).

Breton et al. (2016) studied the effects of a sub chronic exposure to cadmium and lead as risk factors for the development of Inflammatory Bowel Disease. Using peripheral blood mononuclear cells from four healthy donors, the authors exposed the cells to non-toxic doses of cadmium chloride and lead chloride in the presence and absence of concomitant bacterial stimulation. The authors concluded that an exposure to cadmium or lead alone didn't alter basal cytokines release (30).

Two studies used human gut samples to evaluate the importance of inorganic micro- and nanoparticles in the gut ecology. Powell et al. (1996) analysed samples from 20 patients, 10 with Crohn's Disease, 5 with Ulcerative Colitis and 5 with colonic carcinoma. All samples had lymphoid aggregates which is important because macrophages at the base of human gut associated with lymphoid tissue become loaded with a dark granular pigment that is rich in aluminium, silicon and titanium. This situation happens since young age. The study analysed three types of microparticles: Titanium dioxide, aluminosilicates and silicates without aluminium. The authors concluded that the presence of titanium dioxide, aluminium, silicon and iron in high concentrations in macrophages from human gut is associated lymphoid tissue (34).

Gatti (2004) analysed 18 patients, 16 with colon cancer or ulcerative colitis and 2 with Crohn's disease. The controls for comparison were selected from healthy people. To assess the presence of micro and nano-particles in colon an Environmental Scanning Electron Microscope technique was used. The results obtained disclosed that aluminium and silicon is present patients with Crohn's Disease or Ulcerative Colitis, and that these compounds are absent in controls' gut tissue (33).

Overall, the studies evaluating the effects of air pollutants disclose contradictory results. A positive association for Crohn's Disease onset was reported for nitrogen dioxide and for Ulcerative Colitis onset an association with sulphur dioxide was reported. When regarding flares of the disease, the study from Ananthakrishnan (2011) reported the influence of carbon monoxide, nitrous oxide and sulphur dioxide and PM2.5 for Crohn's Disease and Ulcerative Colitis' flares, although Nejad et al. (2016) found a positive association, although not statistically significant between carbon monoxide concentration and number and duration of Ulcerative Colitis flares.

From studies reporting exposure to metals trough drinking water, only Aamodt et al (2008) found a positive association between iron and Crohn's Disease and Ulcerative Colitis. Any other metal reported in these studies didn't have an influence in Inflammatory Bowel Disease

onset. However, some authors reported a higher concentration of copper in serum of patients with Crohn's Disease when comparing to controls. For Ulcerative Colitis' patients, a higher concentration of copper and zinc was present in their serum (28).

Steenland et al. (2013) found a positively increase in the incidence of ulcerative colitis upon exposure to drinking water contaminated with PFOA (18).

When considering exposure trough diet, a positive association between low intake of zinc and the risk of Crohn's Disease was reported (35). For Ulcerative Colitis no association was found. The ingestion of microparticles like titanium dioxide and silicates didn't seem to be associated with Crohn's Disease onset, although a diet with low concentration of titanium dioxide and silicates seem to decrease Crohn's Disease activity index (40,41).

Table 2 – Summary of the studies on the association of environmental contaminants and Crohn's Disease.

Association	Env. Matrices	Biological Matrices	Country	Population	Samples (cases/ control)	Contaminants	Flare/ Onset	Study
Positive Association	Air	-	USA	Patients with IBD flares	2537	Carbon monoxide Nitrous oxide Sulphur Dioxide	Flare	Ananthakrishnan et al (2011)
			Europe	EPICN	190 (38 /152)	Nitrogen Dioxide	Onset	Opstelten el al (2016)
			Europe	THIN	2200 (367/1833)	Nitrogen Dioxide	Onset only in <23 years patients	Kaplan et al (2010)
	Water		Norway	South-eastern regions of Norway	762 (CD= 185)	Iron	Onset	Aamodt et al (2008)
	Diet		UK	Saint Thomas Hospital	18 (9 /9)	Titanium dioxide Aluminosilicates	Flares	Lomer at al (2001)
		Serum	Norway	Patients with IBD from NNGS	47 /123	Copper	Onset	Ringstad et al (1993)
Without Association	Air	-	Europe	EPICN	190 (38 /152)	Particulate matter	Onset	Opstelten el al (2016)
			Europe	THIN	2200 (367/1833)	Nitrous Dioxide Sulphur dioxide Particulate matter	Onset	Kaplan et al (2010)
			Iran	Imam Hospital patients	CD =29	Sulphur Dioxide Carbon monoxide Nitrous dioxide	Flares	Nejad et al (2015)
	Water		Norway	South-eastern regions of Norway	762 (CD = 185)	Aluminium	Onset	Aamodt et al (2008)
			Finland	Populations across Finland	702 (CD = 240)	Aluminium Iron Manganese Ammonium Nitrites	Onset	Lehtinen et al (2016)
	Diet		UK	London Hospital patients with IBD	182 (91/91)	Titanium Dioxide Particulate silicates	Onset	Lomer et al (2004)
	House dust	-	Germany	Munich IBD patients	57 /91	Endotoxin	Onset	Boneberger et al (2011)
Negative Association	Diet	-	USA	NHS I and II	CD = 269	Zinc	Onset	Ananthakrishnan et al (2015)

Table 3 - Summary of the studies on the association of environmental contaminants and Ulcerative Colitis.

Association	Env. Matrices	Biological Matrices	Country	Population	Samples (cases/ controls)	Contaminants	Flare/ Onset	Study
Positive Association	Air	-	USA	Patients with IBD flares	UC = 1353	Carbon monoxide	Flare	Ananthakrishnan et al (2011)
						Nitrous oxide		
						Sulphur Dioxide		
			Europe	EPICN	520 (104/416)	Nitrogen Dioxide	Onset	Opstelten el al (2016)
			Europe	The Health Improvement network	3553 (591/296)	Sulphur Dioxide	Onset only in <25 years patients	Kaplan et al (2010)
	Water		Norway	South-eastern regions of Norway	762 (UC= 435)	Iron	Onset	Aamodt et al (2008)
	Water	Serum	USA	Inhabitants near to DuPont plant	32254	PFOA	Onset	Steenland et al (2013)
		Serum	Norway	Patients with IBD from NNGS	47 /123	Copper	Onset	Ringstad et al (1993)
Without Association	Air	-	Europe	EPICN	520 (104/416)	Particulate matter	Onset	Opstelten el al (2016)
				The Health Improvement network	3553 (591/296)	Nitrous Dioxide	Onset	Kaplan et al (2010)
						Sulphur dioxide		
						Particulate matter		
			Iran	Imam Hospital patients	UC =37	Sulphur Dioxide	Flares	Nejad et al (2015)
						Carbon monoxide		
						Nitrous dioxide		
	Water		Norway	South-eastern regions of Norway	762 (UC= 435)	Aluminium	Onset	Aamodt et al (2008)
			Finland	Populations across Finland	702 (UC= 462)	Aluminium	Onset	Lehtinen et al (2016)
						Iron		
						Manganese		
						Ammonium		
						Nitrites		
						Particulate silicates		
	House dust	-	Germany	Munich IBD patients	23 /91	Endotoxin	Onset	Boneberger et al (2011)
Negative Association	Diet	-	USA	NHS I and II	UC = 338	Zinc	Onset	Ananthakrishnan et al (2015)

Risk of Bias

In this systematic review we considered bias for recruitment strategy, blinding, exposure assessment, confounding, incomplete outcome data, selective report and conflict of interest. We also graded bias in 4 categories: “low risk”, “probably low risk”, “probably high risk” and “high risk”. The conclusion of this analysis for each study included in this systematic research is shown in figure 8.

The global evaluation of risk of bias is presented in figure 9. Globally studies present a low risk of bias. The domain “Blinding” was mostly classified with probably low risk of bias, since most of the studies present low information about sample selection, although there is indirect evidence that participant selection and inclusion/exclusion criteria where consistence across studies.

The domain “Exposure Assessment” was classified with probably high risk since most of studies did not assessed the concentrations of contaminants and used the information available from databases.

The domain “Confounding” was the one with high risk classification since most of the studies simply analysed one environmental matrix for environmental contaminants, without studying the possible interaction with other environmental contaminants from other matrices of exposure, or the same environmental contaminant in other environmental matrices. Also, previous pathologies or exposure to other environmental contaminants or even the same environmental contaminant was not assessed.

When assessing “Conflict of Interests”, the studies where classified with low risk or probably low risk since there is a report on the inexistency of conflict of interest or they do not present sufficient information to conclude that there is a conflict of interest but there is indirect evidence that suggests that the studies were free of support from entities with financial interest in the results obtained.

	Recruitment strategy	*Blinding*	Exposure assessment	Confounding	Incomplete outcome data	Selective reporting	Conflict of Interest
Aamodt et al. (2008) (17)	+ +	++	-	-	++	+	++
Ananthakrishnan et al. (2015) (35)	-	+	--	--	++	++	++
Ananthakrishnan et al. (2011) (37)	++	+	-	--	-	+	++
Boneberger et al. (2011) (27)	++	++	-	--	++	++	++
Breton et al (2016) (30)	-	+	++	--	++	++	+
Gatti (2004) (33)	-	+	++	--	-	++	+
Kaplan et al (2010) (39)	++	++	-	-	-	++	++
Lehtinen et al (2016) (31)	+	+	-	- -	+	++	+
Lomer et al (2001) (41)	+	++	-	-	++	++	+
Lomer et al (2004) (40)	++	+	-	-	++	++	+
Nejad et al (2016) (38)	-	+	--	--	-	+	+
Opstelten et al (2016) (36)	++	+	-	--	-	++	++
Powell et al (1996) (34)	+	+	++	--	-	++	+
Ringstad et al (1993) (28)	++	+	++	-	++	++	++
Steenlend et al (2013) (18)	++	++	-	--	++	+	++
Thilo-körner et al (2000) (29)	++	+	++	-	++	++	++

Figure 8 - Heat map depicting the risk of bias for each individual study. (++ - low risk, + - probably low risk, - - probably low risk, -- -high risk).

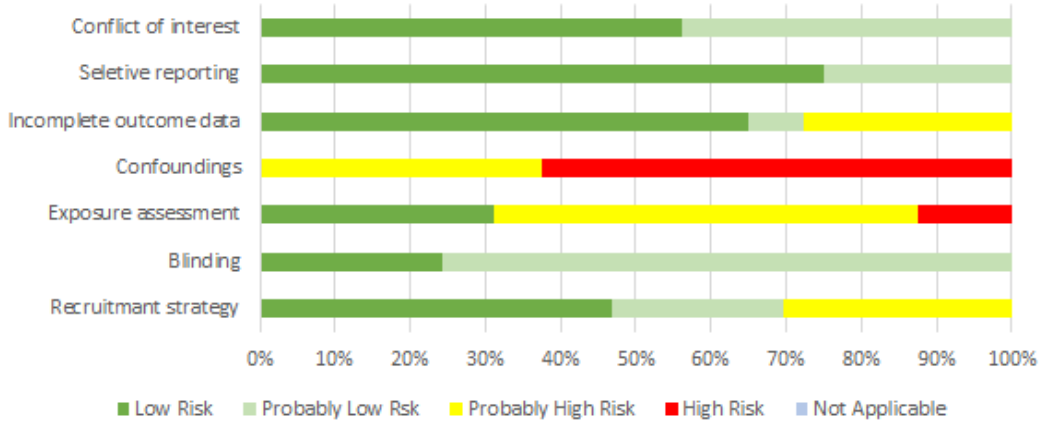

Figure 9 - Relative frequency of the risk of bias across all studies.

Quality of Evidence

The results of the evaluation of the quality of the evidence are shown in Table 4. The quality of the body of evidence was classified as "moderate to low". This result is due to the negative influence of two factors: inconsistency of the evidence and imprecision of the evidence. The "Inconsistency of Evidence" factor underwent a -2 degree evaluation since, in addition to having a great variability of results, conflicting results were also obtained. For example, for the same compounds different studies disclosed different effects. The factor "Imprecision of evidence" was downgraded with -1 since some confidence intervals are wide. The remaining factors do not appear to have negatively influenced the body's quality of evidence. On the other hand, it was not possible to increase their quality since the included studies did not show a magnitude of effects nor a dose-response gradient.

Table 4 - Evaluation of Quality of Evidence.

Factors That Decrease Quality of Evidence	
Risk of Bias	0
Indirectness of evidence	0
Inconsistency of evidence	**-2**
Imprecision of evidence	**-1**
Publication bias	0
Factors that increase quality of evidence	
Large magnitude of effects	0
Dose-response gradient	0
Residual confounders or bias	0
Final assessment of quality	Moderate to low

Strength of evidence

Since there is some inconsistency in the results obtained in this systematic review, we need to rate the strength of evidence as Inadequate evidence on toxicity.

Discussion

An increase in the incidence of Inflammatory Bowel Disease is occurring across the world, this increase is, as previously mentioned, more dramatic in regions that traditionally were not affected by this Disease (7). This fact seems to strengthen the hypothesis that environmental factors may be at play. The aim of this thesis was to understand the actual knowledge about the importance of exposure to environmental contaminants and their influence in Inflammatory Bowel Disease.

This systematic review seeks to clarify the actual knowledge about the relationship between exposure to environmental contaminants and Inflammatory Bowel Disease's onset or fares. An exhaustive research was carried out in Scopus, Web of Science, PubMed/MEDLINE in an attempt to obtain a comprehensive, but at the same time, specific view of the results obtained. After applying the inclusion and exclusion criteria, we finished with 16 studies for this systematic review.

Studies included in this systematic review present a wide range of environmental matrices for exposure and environmental contaminants.

Four studies included in this systematic review analyzed the influence of air pollutants in IBD. These pollutants included: carbon monoxide, nitrous dioxide, sulphur dioxide, and particulate matter. The obtained results are however conflicting: Ananthakrishnan et al. (31) found a correlation between carbon monoxide, nitrous oxide, sulphur dioxide and PM2.5 and an increase in hospitalization rates (incidence rate ratio 1.40; 95% confidence interval 1.31 – 1.50; $p < 0.001$), on the contrary Nejad et al (2016) didn't find any association between exposure to nitrogen dioxide and sulfur dioxide and an Inflammatory Bowel Disease flare.

Nejad et al (2016) report a not statistically significant association between higher concentration of carbon monoxide and the number and duration of Ulcerative Colitis flares flare ($p=0.135$ and $p=0.08$ respectively) (38).

Another important constituent of air pollution is particulate matter. In general, particulate matter can enter the intestine either directly or through the clearance of mucus from the airways. Once in the gastrointestinal tract, especially when in the intestinal tract, the particles will have a direct activity on the epithelial cells of the intestine. Their effects will largely depend on the size of the PM reaching the intestine. For example, for larger particles (<10μm) no associations with the development of Inflammatory Bowel Disease were found however, if smaller particles are considered <2.5μm an association with increasing hospitalization rates was found (incidence rate ratio 1.25; 95% confidence interval 1.18 – 1.33; $p < 0.001$) (36–39).

Iron is the second most abundant metal in the earth's crust. It is one of the most important metals for humans because it is necessary to many of the enzymatic processes and proteins. However, if it is not bound to carrier proteins after absorption, it has corrosive capacities at the level of the gastrointestinal tract and in biological fluids. Thus, this iron toxicity leads to cellular oxidation, resulting in the production of free radicals that affect DNA, leading to mutations and malignant transformations that lead to the onset of diseases (42). From the studies dealing with the ingestion of contaminated water, only one study found a positive association between the metal iron and the incidence of Inflammatory Bowel Disease, Crohn's Disease and Ulcerative Colitis. From the study of Lehtinen et al (2016), they also quantified iron in tap water, although in this study authors didn't observe a positive association between this metal and Inflammatory Bowel Disease (31).

Exposure to aluminium may occur through ingested foods, water, beverages, or drugs. Aluminium has no known biological function and its accumulation can be extremely toxic, especially at the neurological level (42). However, studies indicate that 40% of ingested aluminium accumulates in the intestinal mucosa. A study conducted in the laboratory with mice proved that aluminium has the ability to modulate intestinal inflammation in vivo. The results demonstrated a worsening of colitis, delayed mucosal recovery accompanied by inflammation, specific antigen response by T cells and increased production of inflammatory cytokines. Aluminium has also been shown to have the ability, either alone or with the support of bacteria, to induce the formation of granulomas (43). In the studies of water exposure, aluminium didn't present an association with Inflammatory Bowel Disease (17,31). Although in this systematic review the importance of aluminium was only described by Powel et al (1996) and Gatti (2004).

Cadmium is present in many household and industrial activities and can also result from the combustion of fossil fuels. Its presence is also detected in cigarettes (44). Cadmium is already associated with diseases such as pneumonitis, pulmonary oedema, osteoporosis and cancer, being recognized as carcinogenic to humans by International Agency for research on Cancer (45). The most prevalent are pulmonary and renal diseases (44). Lead is a metal considered highly toxic with a high distribution in the environment. It was present in plumbing tubes, paints, batteries and toys. This metal has the ability to deregulate cell stability, causing oxidative stress. The production of larger amounts of free radicals occurs, such as reactive oxygen species and a decrease in the level of antioxidants. This leads to deregulation in cell adhesion processes, maturation, ion transport, apoptosis and enzymatic dysregulation (42). This metal is related to renal, cardiovascular, renal, haematological, gastrointestinal and reproductive problems. There is accumulation of this metal in the bone (44). Breton et al (2016) related a potential effect of these two metals (cadmium and lead) in the immune system modulation. Although an association was found and human cells were used, we cannot conclude the potential of these metals on Inflammatory Bowel Disease onset, since this evaluation didn't comprise the real gut's physiology (30).

From the only study regarding exposure to perfluorooctanoic acid, a non-biodegradable and persistent environmental contaminant, demonstrated a significant association between perfluorooctanoic acid exposure and the incidence of Ulcerative Colitis(18).

Diet, fundamental for the survival of the human being, is also a vehicle that allows the entrance of numerous compounds with capacity to cause disease in the human organism. These compounds, such as pesticides, mycotoxins or metals, are brought into contact with food during the various processes from production to food intake. These compounds may interfere with the intestinal microbiome and may alter the intestinal balance (46). The studies analysed in this systematic review that evaluated diet as the exposure pathway, concluded that zinc intake was inversely related to the risk of development of Crohn's Disease. In what concerns microparticles like titanium dioxide and silicates, studies show that they don't seem to influence the incidence of Crohn's Disease since the intake is similar in individuals with the disease and in healthy people. When concerning a low intake of titanium dioxide and silicates in patients already having Crohn's Disease, an association between low intake and a decrease in Crohn's Disease activity index was found (35,40,41).

Although some studies addressed the role of metals in IBD, their quantification in biological samples from patients and controls was only performed in only one study. Ringstad et al. (41) quantified the levels of selenium, copper and zinc in serum samples. They concluded that higher concentrations of cooper in serum may be responsible for the development of Crohn's Disease or Ulcerative Colitis. Low serum concentrations of selenium are found in men and women with Crohn's Disease and only in men with Ulcerative Colitis (28).

The effects of micro and nano particles in IBD were also studied. From studies analysing gut samples the one from Powell et al (1996) showed the presence of titanium dioxide, aluminium, silicon and iron in high concentration in macrophages from human gut associated lymphoid tissue, but in this study, no comparison with healthy patients was performed, and thus no conclusions on the importance of these particles on the pathogenesis of Inflammatory Bowel Disease can be drawn. However, Gatti (2004) when using colon tissue found aluminium, and silicon in patients with Crohn's Disease or Ulcerative Colitis, whereas in controls' gut tissue these metals were absent. Although, since the selection of controls didn't mimic the characteristics of the case group, it is not possible to conclude on the relation between these compounds and the onset of Inflammatory Bowel Disease (33,34).

Besides chemical contaminants, the role of biological contaminants in IBD was also studied (28). The endotoxins present in the outer membrane of gram-negative bacteria are pointed to be an influencing factor for the development of asthma or allergies. From the study of Boneberger et al. (2011), no positive association between endotoxin exposure and an increase in the incidence of Inflammatory Bowel Disease was found. In fact, results even show that an inversely related association (adjusted odds ratio 0,70; 95% confidence interval 0,46 – 1,04) between endotoxin exposure and Inflammatory Bowel Disease is possible (27).

When analysing all the studies present in this systematic review, we can only conclude that there is a small number of environmental contaminants that can be associated with Inflammatory Bowel Disease. These are, for Crohn's Disease, titanium dioxide and silicates that can influence disease's activity index and cooper when considering the disease onset. When considering Ulcerative Colitis carbon monoxide seem to be a responsible factor for disease flares as well as perfluorooctanoic acid.

All the others environmental contaminants studied in this systematic review didn't show a consistent influence on Inflammatory Bowel Disease onset or flares.

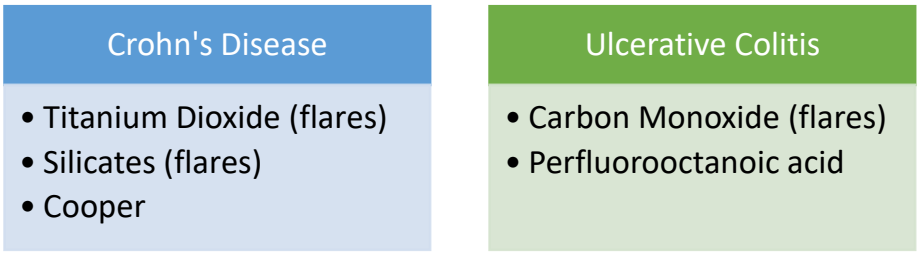

Figure 10 - Environmental Contaminants that have a positive effect on Inflammatory Bowel Disease in the studies analysed.

Overall, in this systematic review, we identified different classes of environmental contaminants, but studies analysing the real concentration of environmental contaminants in biological matrices are rare and the wide range of environmental matrices (air, water, diet) make it difficult to extract valid conclusion related to the evidence of these contaminants in Inflammatory Bowel Disease onset or flares. "Confounding" where mostly rated with High risk since most of the studies simply analysed one environmental matrix for environmental contaminants, without studying the possible interaction with other environmental contaminants from other matrices of exposure, or the same environmental contaminant in others environmental matrices. Also, previous pathologies or exposure to other environmental contaminants or even the same environmental contaminant was not assessed.

Therefore, I suggest that in the future, studies that evaluate the possible association between exposure to environmental contaminants and Inflammatory Bowel Disease populations, with robust sample sizes, and addressing all possible confounding factors should be conducted. Since there is a continuous interaction between the individual and the environment we need to look to possible interactions between different contaminants as causes responsible for Inflammatory Bowel Disease and not only a particular exposure.

Conclusion

Pathophysiology of Inflammatory Bowel Disease is still mostly unknown, but a lot of effort it being made to understand where we can intervene to change or prevent the course of this disease. It is known that the intestinal microbiome and immune system are important parts in the disease development (12,47). Factors that could trigger a change in the normal environment of the microbiome and immune system are still being investigated. Given the sharp increase in incidence and prevalence of Inflammatory Bowel Disease in countries where Inflammatory Bowel Disease was not common, the hypothesis that environmental factors may be responsible for IBD is gaining strength (6). Herein we systematically reviewed the available literature and given the limited number of studies there is still inadequate evidence that environmental contaminants are associated with IBD.

For future studies, protocols that include more than one environmental contaminant could be the next step for a new approach to the pathogenesis of Inflammatory Bowel Disease.

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