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Ruolo della terapia immunosoppressiva e biologica nelle IBD pediatriche

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Summary

Part I: Introduction

Part II: Investigation

Part I: Introduction

1. Pathogenesis of IBD

Inflammatory bowel diseases (IBD), Crohn disease, ulcerative colitis and indeterminate colitis, are immune-mediated disorders resulting in chronic, relapsing inflammation of the gastrointestinal tract associated, in some cases, to extraintestinal manifestations. These subtypes are characterized by inflammation of the digestive tract, with CD involving widespread inflammation of all layers of the GI tract while UC is characterized by localized inflammation of the colon. Current modalities for the diagnosis of both the subtypes involve a combination of invasive endoscopic procedures and diverse clinical indices that provide only relative measures of disease severity and outcome.

In genetically predisposed individuals, environmental factors and abnormal immune responses to some components of intestinal bacteria eventually trigger chronic inflammation and mucosal injury.

1.1 Genetic Issues

The importance of genetic components in the pathogenesis of IBD is suggested by:

- Ethnic variations: highest rates in Caucasian individuals, especially of Jewish descent;
- Family aggregation, stronger for CD;
- Twin studies, with 36% concordance for CD and 16% for UC.

Identifiable gene variants are NOD2/CARD15, OCTN1 and OCTN2, DLG5, HLA polymorphisms among others.

The NOD2/CARD15 gene located on the IBD1 locus on chromosome 16 predisposes to CD (but not UC); several polymorphisms are known. NOD proteins are recognition receptors which serve as bacterial sensing molecules, binding muramyl dipeptide and activating inflammatory cascade (Fig.1). Possessing one NOD2/CARD15 allele confers a two-three fold relative risk of developing CD; this risk rises to 17-fold when two alleles are present.

Analyses of adult population have demonstrated that carriage of NOD2/CARD15 risk alleles predicts disease onset at an earlier age, ileal disease location in a dose-dependent manner, fibrostenosing behaviour of disease and family history of IBD. Among pediatric population, 20-

65% of CD patients possess at least one NOD2/CARD15 mutation, though there is less evidence of an association with fibrostenosing disease.

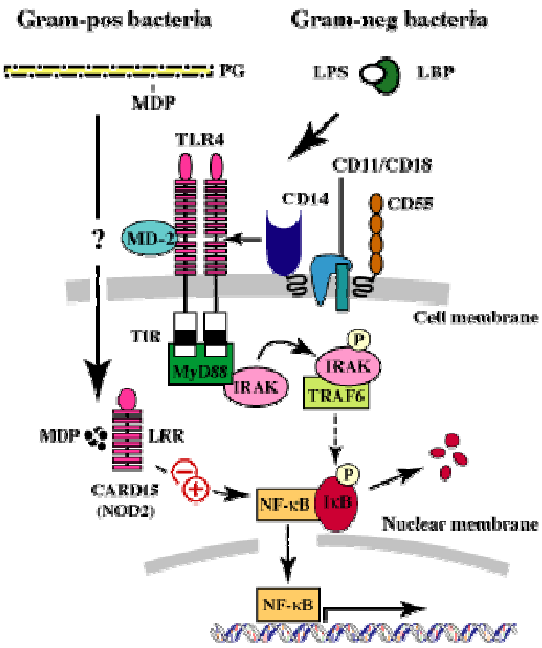

Figure 1: immune patterns of bacterial recognition in the intestine.

Recently, genome-wide association studies helped identifying and understanding other potential predisposing alleles in this patients. Genome-wide association studies meta-analysis has led to confirmation of more than 70 genes or loci that confer susceptibility to CD and 47 to UC, mostly in adult populations *(Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, et al. Genomewide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008;40: 955–26).* In particular, genome-wide association and replications studies identified gene variants, including protein tyrosine phosphathase nonreceptor type 2 (PTPN2), NK2 transcription factor related locus 3 (NKX2-3), and tumor necrosis factor superfamily member 15 (TNFSF15) *(Wellcome Trust Case Control Consortium . Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;47: 61–87).* A genome-wide association study carried out in pediatric-onset IBD patients identified two novel IBD loci located on chromosome 20q13 and 21q22, close to the tumor necrosis factor receptor superfamily member 6B (TNFRSF6B) and proteasome-assembling chaperone 1 (PSMG1) genes *(Kugathasan S, Baldassano RN, Bradfield JP, Sleiman PM, Imielinski M, et al. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. Nat Genet 2008;40: 1211–1215).*

A recent Italian study investigated a cohort of 2283 adult and pediatric patients (the author contributed to recruit) confirming these results and finding a significant association with pediatric IBD cohort with 6 variants, namely PTGER4, TNFSF15, NKX2-3, ZNF365, IFNG, and PSMG1 *(Latiano A, et al. Investigation of Multiple Susceptibility Loci for Inflammatory Bowel Disease in an Italian Cohort of Patients. PLoS ONE 2011:6: e22688).*

1.2 Immune Response and Inflammation

The cellular barrier of the gastrointestinal tract is formed by a continuous single layer of epithelial cells sealed with tight junctions, whose competency lies upon the regulation of proteins such as occluding, claudins and junction-adhesion molecules. On the luminal surface of these cells, a layer of mucus, secretory IgA, antibacterial proteins (defensins) and enzymes form the first line of defense against pathogen invasion. Nonetheless, a controlled transport of selected luminal molecules through M cells into the underlying lymphoid follicle occurs. M cells are highly specialized epithelial cells that express fewer microvilli, low alkaline phosphatase activity, and no IgA secretory component. Transported antigens are then captured by underlying dendritic cells, which present antigenic peptides to interfollicular T cells within the mesenteric lymph node. There is evidence that abnormal intestinal permeability occurs among CD patients and their healthy first-degree relatives, and this abnormality may predispose to excessive antigen uptake, continuous immune stimulation, and eventually mucosal inflammation. Genetic and environmental factors may explain epithelial barrier dysfuncion (i.e. smoking, non-steroidal antinflammatory drugs, OCTN and DLG-5 mutations).

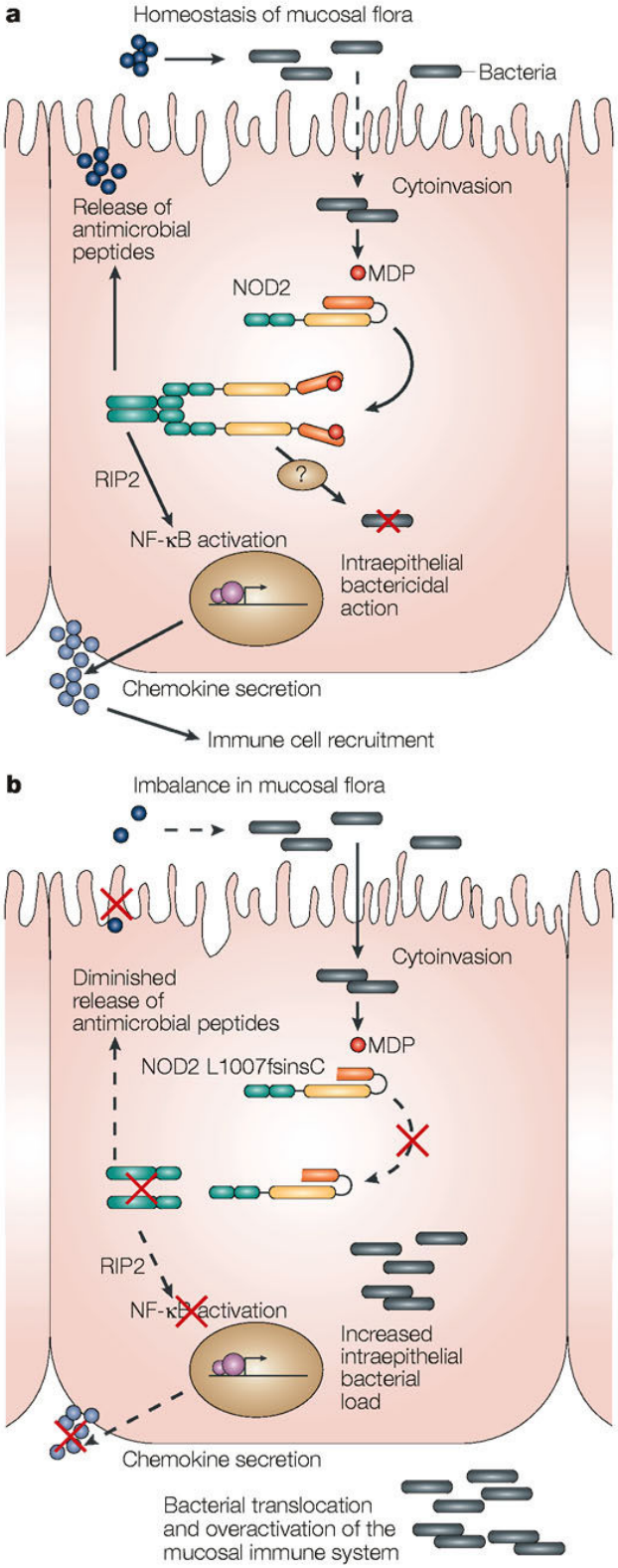

Figure 2: homeostasis (a) and imbalance (b) of intestinal mucosal flora.

The immune system can be divided into **innate** and **adaptive** immunity, which differ in terms of specificity and timing of response. The first is a rapid responding system, working through membrane-bound (Toll-like receptors - TLRs) or cytoplasmic receptors (Nucleotide-binding Oligomerization Domain family members – NODs), and several cells: macrophages, neutrophils, eosinophils, specific lymphoid cells such as natural killer T cells. Dendritic cells induce adaptive responses either in a tolerogenic or in an immunogenic fashion, acting in mesenteric lymph nodes and expressing different types of cytokins and co-stimulatory molecules. It is established that TLRs and NODs pathways lead to activation of NF-kB responsive genes mediating inflammatory responses.

The adaptive immune system comprises CD4+ and CD 8+ T cells lying in the lamina propria, T-regulatory cells, B cells. T cells antigenic expression and interaction with other cell types (mainly endothelium) regulates circulation (selectines – CD-34), crossing of vessel wall (LFA-1 – ICAM 1-2), activation or anergy (TCR – stimulatory and co-stimulatory molecules), gut homing (CCR9/α4β7 – MadCAM1), and assumption of different phenotypes.

Specifically, T helper 1 phenotype refers to IFNγ-producing cells, effective in controlling intracellular pathogens; T helper 2 phenotype include IL-4, IL-5 and IL-13 producing cells, effective in parasitic infections and allergic responses; T helper 17 phenotype: IL-17 and IL-6 producing cells, responsible for acute inflammation and granulocytes recruitment.

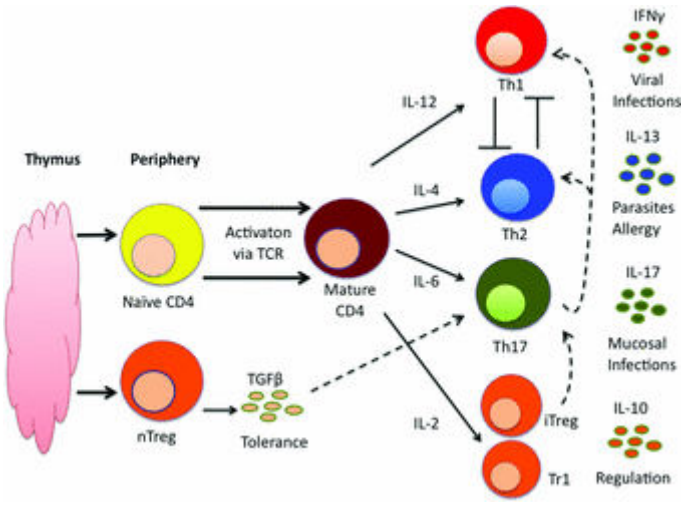

Figure 3: lymphocyte differentiation patterns.

T-regulatory cells consist of three main subtypes: CD4+CD25+ cells, Tr1 cells producing IL-10 and TGF-β, and Th3 cells producing TGF-β and derived through oral tolerance *(Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity 2006;25:195-201).*

B cells of the GALT (gut associated lymphoid tissue) enhance mucosal defense secreting IgA and IgM (approximately 3 grams of sIgA per day - according to *Conley ME, Delacroix DL. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? Ann Intern Med 1987;106:892-899).* Over 80% of all human plasma cells can be found in the gut, and nearly all these cells produce IgA. The joining ("J") chain of polymeric IgA interacts with the polymeric Ig receptor expressed on the basolateral surface of epithelial cells facilitating exportation of thesIgA to the gastrointestinal lumen, where sIgA coat commensal and pathogenic bacteria in order to promote M-cell mediated bacterial uptake and presentation to intestinal dendritic cells. Organized lymphoid tissue of the small bowel (Peyer's patches) consists of B cell follicles and CD4+ and CD8+ T cells. The number of Peyer's patches increases from 80-120 at birth to 250 by adolescence *(Cornes JS. Peyer's patches in the human gut. Proc R Soc Med 1965;58:716).*

In human IBD, CD shows the major features of a Th1 response pattern, while UC represents an atypical Th2 response *(Monteleone G, et al. Interleukin12 is expressed and actively released by Crohn disease intestinal lamina propria mononuclear cells. Gastroenterology 1997;112:1169-1178; Fuss IJ, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J Clin Invest 2004;113:1490-1497).*

Intestinal epithelial cells express membrane-bound (e.g. TLR) as well as intracellular (e.g. NOD) receptors to sense gut microbes, and encounter commensal and potentially pathogenic bacteria routinely balancing tolerance and immune responses to them. Bacteria may contact immune cells of the GALT in three different ways:

1. Infected epithelial cells may undergo apoptosis, and cellular fragments containing bacteria may be ingested by macrophages and dendritic cells and subsequently be presented to T cells through MHC class II-TCR interaction and/or CD8+ cytotoxic T cells; co-stimulatory molecules expressed by antigen presenting cells play a very important role in determining the response type (tolerance versus inflammation);
2. Dendritic cells and macrophages may acquire bacteria directly from the environment and kill them, and then present bacterial antigens on MHC class II to CD4+ cells;
3. Lymphocytes may contact bacterial antigens directly in the absence of antigen presenting cells.

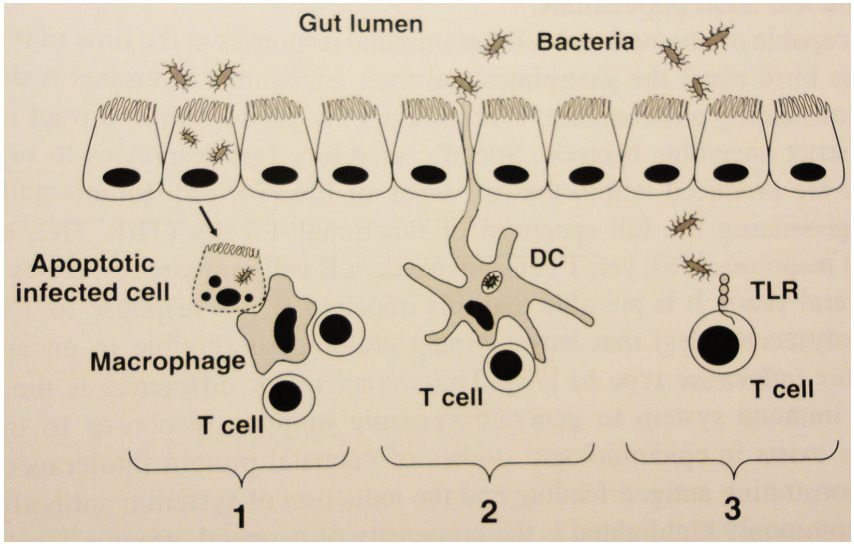

Figure 4: immune recognition of intestinal microbes.

Following these multifactorial antigen-driven immune response, a series of secondary events occur, including the production of proteolitic enzymes (matrix metalloproteinases, oxygen reactive species), eventually leading to structural tissue damage and necrosis.

Differences have been observed between neonatal and adult immune responses.

Innate immune responses are mostly germline-encoded through recognition of microbial ligands by pathogen recognition receptors; on the other hand, adaptive responses are antigen-specific and thus require post-natal exposure to dietary and microbial antigen to develop immunologic memory. In humans, within hours from birth the gastrointestinal tract is colonized with facultative and strictly anaerobic bacteria, triggering the production of specific secretory IgA responses *(Mackie RI, et al. Developmental microbial ecology of the neonatal intestinal tract. Am J Clin Nutr 1999;69:1035S-1045S).* Moreover, infants may generate the full spectrum of T cells and T-cell dependent B cell responses, while T-cell independent B cell responses take years to reach full maturity *(Fadel S, Sarzotti M. Cellular immune responses in neonates. Int Rev Immunol 2000;19:173-193).*

Other important aspects are the tendency of the young mucosal immune system to generate immune responses to oral antigens and the propensity of neonatal effector T cell responses to be preferencially Th2 polarized *(Rowe J, et al. Antigen-specific responses to difteria-tetanus-acellular pertussis vaccine are initially Th2 polarized. Infect Immun 2000;68:3873-3877).* The latter may represent a "default" pathway aimed at keeping inflammatory reactions controlled at an early stage of life, when Th1 polarization could lead to inflammatory damage.

In experimental (animal) models of IBD, switches from a Th1 to a Th2 response pattern have been observed; this means that, even though the clinical manifestations of the disease remain

constant, the underlying immunopathology may change over time *(Spencer DM et al. Distinct inflammatory mechanisms mediate early versus late colitis in mice. Gastroenterology 2002;122:94-105).* Therefore, immunomodulators that are effective in early disease may no longer be effective in chronic disease. In humans, it has been noted that children with early onset Crohn disease have a significant longer remission in response to TNF-α blockade than children with late disease *(Kugathasan S et al. Prolonged duration of response to infliximab in early but not late pediatric Crohn disease. Am J Gastroenterol 2000;95:3189-3194),* and this could indicate that even in humans the cytokine secretory pattern of mucosal T cells can be better modulated early in the course of the disease than later on. Further clinical observations are needed to confirm this hypothesis.

1.3 The Role(s) of Cytokines

In animal and human models, inflammation may be triggered by either Th1 or Th2 T cell responses; the former is characterized by the secretion of interleukin (IL)-1, IL-2, IL-6, IL-12, IL-18, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α), while the latter by increased levels of IL-4, IL-5, IL-10, IL-13. *(Neurath MF, et al. Experimental granulomatous colitis in mice is abrogated by induction of TGF-beta-mediated oral tolerance. J Exp Med 1996;183:2605-2616. Reinecker HC et al. Enhanced secretion of tumor necrosis factor-alpha, IL-6 and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn disease. Clin Exp Immunol 1993;94:174-181).* Cytokines can affect the synthesis or secretion of reactive oxygen species, nitric oxide, leukotrienes, platelet-activating factor, and prostaglandins. Pro- and anti-inflammatory responses are required to maintain the integrity of the intestinal mucosa due to the environment in which it exists. In facts, the intestinal mucosa constantly faces innocuous and harmful antigens from food, commensal and pathogenic bacteria; therefore, the immune system must be able to regulate itself either by the action of regulatory cells or by the action of cytokines such as IL-4, IL-5, IL-10, TGF-β, IL-1, and TNF-α.

Pro-inflammatory cytokines are TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-12, IL-23, IL-17, IL-18, IL-13, while TGF-β , IL-4, IL-10 are anti-inflammatory cytokines.

TNF-α is secreted by macrophages, monocytes, neutrophils, TCD4+ cells and NK cells in response to stimulation by bacterial lipopolisaccharides, interferons, IL-2 and granulocyte macrophage colony stimulating factor (GM-CSF). Its production is inhibited by IL-6 and TGF-β. TNF-α is an agonist of the p38 and c-jun N terminal kinase cascades, two important signaling

pathways of the mitogen activated protein (MAP) family involved in the generation of the inflammatory responses. *(Shetty A, Forbes A. Pharmacogenomics of response to anti-tumor necrosis factor therapy in patients with Crohn disease. Am J Pharmacogenomics 2002;2:215-221)* In non-active macrophages, TNF-α induces the synthesis of IL-1 and prostaglandin E2, resulting in the potentiation of the inflammatory cascade activation. TNF-α can also enhance the proliferation of T cells induced by various stimuli, and activate osteoclasts inducing bone resorption. Although TNF-α is required for normal host immune responses, the overexpression can have severe pathologic consequences. In particular, TNF-α knockout mice do not develop significant colitis, while in experimental IBD treatment with TNF-α antagonists ameliorates intestinal inflammation *(Neurath MF, et al. Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice. Eur J Immunol 1997;27:1743-1750).* Human studies confirmed these results *(Targan SR, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn disease. N Engl J Med 1997;337:1029-1035).* However, TNF-α mediates a part of the cell-mediated immunity against bacteria and parasites by stimulation of phagocytosis and the synthesis of superoxide dismutase in macrophages, conferring protection against Listeria monocytogenes and Mycobacterium tuberculosis infections; infection sustained by these organisms is a possible risk for patients treated with TNF-α antagonists.

Interferon gamma is produced mainly by CD4+, CD8+ T lymphocytes and natural killer cells. It is an immunomodulatory cytokine, and it stimulates T-cell growth and differentiation and the expression of MHC class molucules; in concert with TNF-α, IFN-γ contributes to tissue destruction. Fontolizumab, a humanized monoclonal antibody, has been studied in IBD patients, showing low efficacy in inducing remission *(Reinisch W, et al. A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanized anti-interferon gamma antibody, in patients with moderate to severe Crohn disease. Gut 2006;55:1138-1144).*

IL-1 consists of two subunits (α and β) which are produced by monocytes, macrophages and endothelial cells. This subunits act either locally and systemically promoting inflammation, stimulating T-helper to produce IL-2, promoting B-cells proliferation and synthesis of immunoglobulins, and activation of natural killer cells, fibroblasts and thymocytes.

IL-1 also promotes the adhesion of neutrophils, monocytes, T- and B-cells by enhancing the expression of adhesion molecules (ICAM-1, ELAM), and activates osteoclasts. IL-1 receptor antagonist is produced by intestinal epithelial cells and is considered to be one intestinal mechanism to down-regulate the immune response.

IL-2 promotes T-cell proliferation binding to CD25, and is produced mainly by CD4+ T lymphocytes following activation (not by resting cells). It is a growth factor for all subpopulations of T-lymphocytes, including suppressive T regulatory cells.

IL-6 is an important cytokine in inflammation, regulation of T cell responses and apoptosis; it is produced mainly by monocytes, fibroblasts, endothelial cells, macrophages, T- and B-lymphocytes. IL-6 is a B-cell differentiation factor and an activating factor for T-cells; together with TGF-β, IL-6 can induce the development of Th17 cells. IL-6 plasmatic levels were found increased in IBD patients compared with controls; humanized anti-IL-6R antibody (tocilizumab) is under investigation for treatment of Crohn disease.

IL-12 is a proinflammatory cytokine that is important in the differentiation of naïve T-cells into CD4+ Th1 cells (producing IFN-γ). IL-12 is secreted by monocytes, macrophages, dendritic cells, NK cells in response to bacterial and parasitic products. IL-12 is increased in the intestine of CD patients *(Fuss IJ, et al. Both IL-12p70 and IL-23 are synthetized during active Crohn disease and are down-regulated by treatment with an anti-IL12p40 monoclonal antibody. Inflamm Bowel Dis 2006;12:9-15)*, and treatment with an anti-IL-12 monoclonal antibody is being evaluated for IBD patients.

IL-23 is a proinflammatory cytokine that shares structural homology with IL-12. It is produced by activated dendritic cells and macrophages, and promotes the differentiation of Th17 cells.

IL-17 is secreted by a subset of CD4+ T-cells called T-Helper-17, monocytes and neutrophils and induces the production of several cytokins promoting inflammation (IL-6, G-CSF, GM-CSF, IL-1β, TGF-β, TNF-α, IL-8, PGE2 among others). IL-17 binds to the IL-17 receptor on endothelial cells and epithelial cells and subsequently promotes recruitment of inflammatory cells to the site *(Kolls JK, Linden A. Interleukin-17 family mambers and inflammation. Immunity 2004;21:467-476).* Increased expression of IL-17 has been detected in the intestinal mucosa of patients with IBD *(Fujino S, et al. Increased expression of interleukin-17 in inflammatory bowel disease. Gut 2003;52:65-70).*

The ability to down-regulate or stop inflammation is essential to avoid damage deriving from the immune response. T-regulatory cells, B-lymphocytes, NK-cells and dendritic cells secrete TGF-β, IL-10, interferon-α/β and prostaglandin J2.

TGF-β is produced by a wide variety of lymphoid and non-lymphoid cells, and five isoforms exist. TGF-β can act in autocrine and paracrine modes to control the differentiation, proliferation and state of activation of immune cells, and can inhibit the production and response to cytokines associated with CD4+Th1 and Th2 cells.

IL-4 is produced mainly by Th2 cells and acts in the intestine, where it promotes the proliferation and differentiation of B-cells and the expression of major histocompatibility complex class 2 antigens.

IL-10 is produced by CD8+ cells and by T-helper CD4+ cells after both antigen-specific and polyclonal activation. IL-10 down-regulates the inflammatory response in multiple ways, i.e by reducing the secretion of cytokines (IL-2, IFN-γ and TNF-α), inhibiting antigen presentation and the chemotactic response of CD4+ cells in response to IL-8.

2. Epidemiology and Clinical Features

The incidence of IBD is around 5/100.000/year, with an increasing trend. Northern latitudes populations have a higher predisposition of IBD than southern ones. Suggested risk factors are smoking and the presence of intestinal flora, while appendectomy reduces risk of UC by 69%.

About 25% of IBD occur before the age of 18, with a peak in adolescence, and 4% of pediatric cases occur before the age of 4. Younger children have more isolated colonic disease, and specifically UC, IC or Crohn colitis, while older children have more diffuse disease.

The most common symptoms at presentation are abdominal pain, diarrhea, weight loss, fever, and bloody stools in UC. Unique features in pediatric ages are growth retardation and pubertal delay. Laboratory analyses may show anemia, leukocytosis, thrombocytosis, elevated C-reactive protein and erythrocyte sedimentation rate, hypoalbuminemia, elevated transaminases, elevated anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn disease, elevated perinuclear anti-nuclear cytoplasmic antibody (p-ANCA) in ulcerative colitis. Stool cultures are taken to exclude infections (especially by E. coli, Salmonella, Shigella, Campylobacter, Mycobacterium, Yersinia species).

Diagnosis is based on colonoscopy with biopsy and radiographic imaging of small bowel; many clinicians perform an esophagogastroduodenoscopy (EGDS) with biopsy, which can add important informations. In most cases of Crohn disease, the inflammation is limited to the ileum, cecum and ascending colon, while ulcerative colitis typically affects the rectum and extends proximally in a diffuse continuous distribution without involving the small bowel. Typical CD lesions are aphtae, deep fissures and cobblestoning of the mucosa, while UC is characterized by superficial inflammation, granularity and friability. Furthermore, segmental distribution, ileal stenosis or ulceration, perianal disease, and granulomatous inflammation suggest Crohn disease, while UC usually present continuous distribution with variable proximal extension of the colon.

Histologically, CD present transmural involvement, with ulcers, granulomas, focal changes and patchy distribution, while UC shows mucosal involvement, Goblet cells depletions, crypt distortion and abscesses. In a subgroup of patients with UC, atypical features could be present, whoch do not exclude the diagnosis of UC: ileal inflammation (backwash ileitis), mostly in those with pancolitis; oral ulcers; growth impairment; gastritis; relative rectal sparing; patchiness (areas of normal mucosa between areas of colonic inflammation).

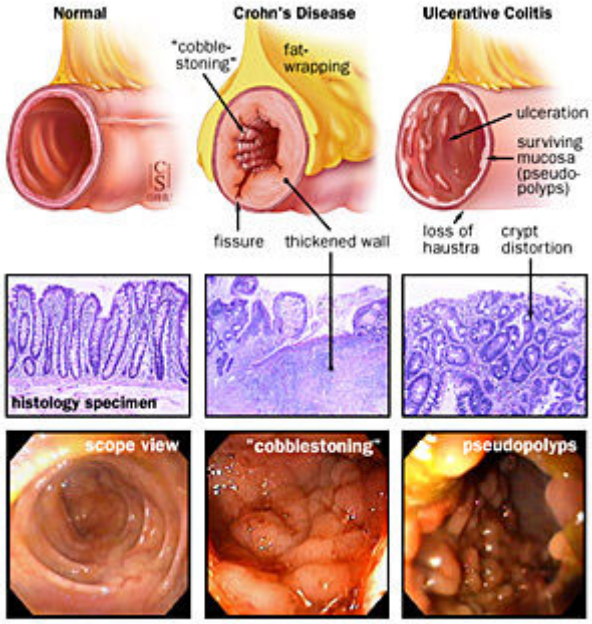

Figure 5: different aspects of Crohn disease and ulcerative colitis.

Classical radiographic imaging study is the small bowel follow through, which can demonstrate narrowing, ulcerations, stenoses, fistulas.

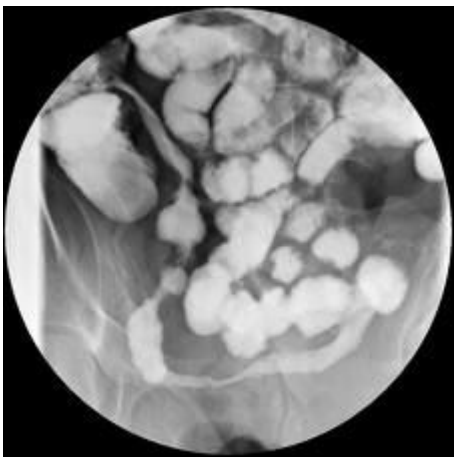

Figure 6: Small bowel follow-through showing CD stenoses.

Other emerging tests are abdominal MRI (useful in assessing disease activity and abdominal complications) *(Friedrich C, et al. Magnetic resonance enterography with and without biphasic contrast agent enema compared to conventional ileocolonoscopy in patients with Crohn's disease. Inflamm Bowel Dis 2012; doi: 10.1002/ibd.22843),* ultrasound with or without contrast (which can evaluate disease activity and does not expose patients to radiations) *(Strobel D, et al. Diagnostics in inflammatory bowel disease: ultrasound. World J Gastroenterol 2011;17:3192-3197),* video capsule endoscopy *(Long MD, et al. Impact of capsule endoscopy on management of inflammatory bowel disease: a single tertiary care center experience. Inflamm Bowel Dis 2011;17:1855-1862).*

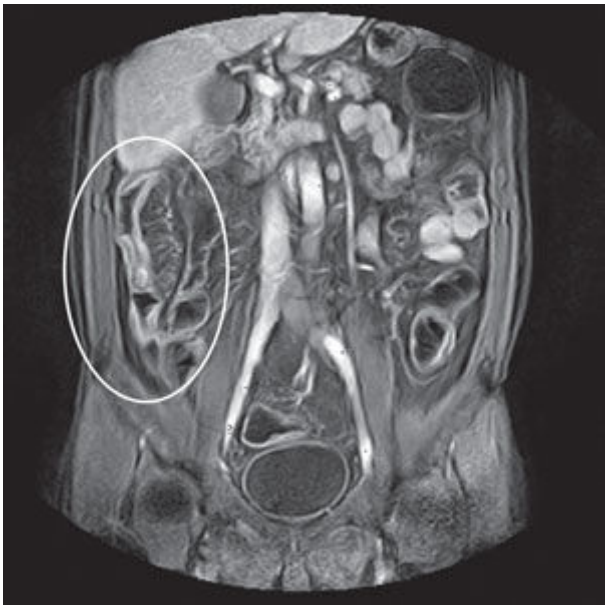

Figure 7: abdominal MRI showing CD activity.

Perianal disease can be studied performing MRI, endoscopic ultrasound and examination under anesthesia (EUA); the most accurate results are obtained when any two out of the three techniques are used *(Schwartz DA, et al. A comparison of endoscopic ultrasound, magnetic resonance imaging, end exam under anesthesia for evaluation of Crohn perianal fistulas. Gastroenterology 2001;121:1064-1072).* Perianal disease ranges from mild lesions (small, non-inflamed skin tags and fissures) requiring only conservative therapy to severe complications (ulcerations, abscesses, fistulas, strictures) requiring aggressive therapy, including surgery. Perianal fistulas were found in 13% of pediatric patients in one study *(Tolia V. Perianal Crohn's disease in children and adolescents. Am J Gastroenterol 1996;91:922-926).*

The Montreal working group proposed UC and CD classification as follows:

- UC: proctitis; left side UC, extensive UC;
- CD: classified based upon A) age at diagnosis: < 16 years; 17-40 years; > 40 years;
  L) location: ileal; colonic; ileocolonic; upper GI;
  B) behavior: inflammatory; stricturing; fistulizing.

Age at diagnosis	Location	Behaviour
A1: below 16 yrs	L1: ileal	B1: inflammatory
A2: between 17 - 40yrs	L2: colonic	B2: stricturing
A3: above 40 yrs	L3: ileocolonic	B3: penetrating
	L4: isolated upper GI disease *	p: perianal disease modifier #

* L4 is a modifier that can be added to L1-L3 when concomitant upper gastrointestinal disease present is present
# "p" is added to B1-B3 when concomitant perianal disease is present

Figure 8: Montreal classification of Crohn disease.

While acute weight loss is common on children with CD and UC, linear growth impairment is more frequent among CD patients. While catch-up growth is often possible with adequate treatment of IBD, up to 35% of young adults diagnosed with CD during childhood have final heights that are significantly shorter than expected *(Markowitz J, et al. Growth failure in pediatric inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1993;16:373-380).*

According to one study, the cumulative incidence of surgery among pediatric Crohn disease patients is 17% at 5 years and 28% at 10 years, and younger age at diagnosis has been associated with decreased risk for surgery *(Gupta N, et al. Risk factors for initial surgery in pediatric patients with Crohn disease. Gastroenterology 2006;130:1069-1077);* in adults, the cumulative incidence of surgery 10 years after diagnosis ranges between 40 and 70%. Patients with ulcerative colitis are more likely to have severe disease, including pancolitis, compared with adults, requiring surgery in up to 20% of cases in the first years of disease *(Stordal K, et al. Pediatric inflammatory bowel disease in southeastern Norway: a five year follow-up study. Digestion 2004;70:226-230).* In children, the overall rate of post-operative clinical recurrence is estimated to be 50% at 5 years after intestinal resection *(Griffiths AM: Factors that influence the postoperative recurrence of Crohn's disease in childhood. In Inflammatory Vowel Disease and Celiac Disease in Children.*

Hadziselimovic F, Herzog B, Burgin-wolff A (Eds). Boston: Kluwer Academic Publishers, 1990, pp. 131-136).

The risk of colon cancer in UC or small intestinal and colon cancer in CD is increased in patients with long disease duration; surveillance colonoscopy is recommended for all children with disease of 10 years duration. Among CD patients, there is an increased relative risk of colorectal cancer of 2.5 (especially for patients with colonic disease), and an increased risk of small bowel cancer of 18-40 *(Jess T, et al. Risk of intestinal cancer in inflammatory bowel disease: a population-based study from Olmsted County, Minnesota. Gastroenterology 2006;130:1039-1046)*; there may also be an increase in risk of developing lymphoma, about fourfold in patients treated with azathioprine/6mercaptopurine. Infliximab may add an additional risk of developing hepatosplenic T-cell lymphoma.

The natural history of Crohn disease is characterized by recurrent exacerbations intersped with periods of inactive disease.

Pancolitis is more frequent in children, while descending colon or distal involvement (proctitis/proctosigmoiditis), is more frequent in adults; moderate to severe activity is at presentation is common in children. About 20% of children at presentation will have severe disease and require intravenous corticosteroids *(Hyams J, et al. The natural history of corticosteroid therapy for ulcerative colitis in children. Clin Gastroenterol Hepatol 2006;4:1118-1123).* About 43% of children presenting with UC have mild disease and 57% have moderate to severe disease. After five years, 9% of children presenting with mild disease and 26% presenting with moderate/severe disease underwent colectomy in one study *(Hyams J, et al. Clinical outcome of ulcerative colitis in children. J Pediatr 1996;129:81-88).* Severe colitis is defined as more than six bloody stools daily and evidence of toxicity such as fever, tachycardia, anemia or an elevated erythrocyte sedimentation rate *(Kornbluth A, Sachar DB. Ulcerative colitis: practice guidelines in adults (update). Am J Gastroenterol 2004;99:1371-1385);* fulminant disease is defined as passing more than 10 bowel movements daily, continuous bleeding, toxicity, abdominal tenderness and distention, need for blood transfusions and evidence for colonic dilatation on a plain film of the abdomen. More severe disease at diagnosis is associated with a higher colectomy rate (9%) at one year than is mild disease at presentation (1%) *(Hyams JS, et al. Clinical outcome of ulcerative colitis in children. J Pediatr 1996;129:81-88)*. Mesalamine is an effective maintenance therapy for mild to moderate UC; corticosteroids are commonly used in moderate to severe disease. Multi-drug resistance gene (MDR-1) may play a role in determining response to steroids *(Farrell RJ, et al. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology 2000;118:279-288).* Other therapeutic options are:

azathioprine, useful in maintaining remission *(Kader HA, et al. Experiences with 6-mercaptopurine and azathioprine therapy in pediatric patients with severe ulcerative colitis. J Pediatr Gastroenterol Nutr 1999;28:54-58);* cyclosporine for inducing remission in severe ulcerative colitis *(Treem WR, et al. Cyclosporine for the treatment of fulminant ulcerative colitis in children. Immediate response, long-term results, and impact on surgery. Dis Colon Rectum 1995;38:474-479);* infliximab to induce and maintain remission *(Russell GH, et al. Infliximab is effective in acute but not chronic childhood ulcerative colitis. J Pediatr Gastroenterol Nutr 2004;39:166-170).* Cyclosporine is effective at inducing remission in up to 80% of children with severe colitis, but by one year most require colectomy *(Treem WR, et al. Cyclosporine for the treatment of fulminant ulcerative colitis in children. Immediate response, long.term results, and impact on surgery. Dis Colon Rectum 1995;38:474-479).* The management of fulminant ulcerative colitis hinges on the response to initial treatment with corticosteroids *(Fabio WA. Step-up versus top-down: application of new biological agents in pediatric inflammatory bowel disease. Clin Gastroenterol Hepatol 2006;4:1094-1096)*; by day 5 one has to consider other therapies such as cyclosporine or infliximab if the patient is not responding to steroid treatment, because extending treatment increases the risk of complications without improving the chance for remission *(Werlin SL, Grand RJ. Severe colitis in children and adolescents: diagnosis, course, and treatment. Gastroenterology 1977;73:828-832).*

Patients with indeterminate colitis have primarily colonic disease involvement, but several clinical, anatomic, endoscopic, or histologic features such as nonspecific gastritis, ileal inflammation or minor ulceration, rectal sparing, or growth failure make the diagnosis of ulcerative colitis uncertain. According to some authors, IC is not a distinct disease entity but rather represents a provisional descriptive term used until the true nature of the patient's underlying type of IBD becomes apparent, usually within a few years following diagnosis *(Odze RD. Pathology of indeterminate colitis. J Clin Gastroenterol 2004;38:S36-S40).*

Assessment of disease severity relies upon clinical, laboratory and endoscopic evaluations. For adult CD patients, the CDAI (Crohn disease activity index) is a combination of clinical symptoms, IBD-related complications, physical examination findings, laboratory tests, weight, and the use of medications to treat diarrhea *(Best WR, et al. Development of a Crohn disease activity index. Gastroenterology 1976;70:439-444).* The pediatric Crohn disease activity index (PCDAI) incorporates patient symptoms, physical exam findings, laboratory parameters and growth measures *(Hyams J, et al. Evaluation of the pediatric Crohn disease activity index: a prospective multicenter experience. J Pediatr Gastroenterol Nutr 2005;41:416-421).*

For adult UC patients, the most commonly used index is the Mayo-Clinic score, which incorporates both clinical and endoscopic evaluations. In children the Pediatric UC Activity Index (PUCAI) has recently been introduced; it is a clinical-based item which is being increasely used in clinical practice *(Turner D, et al. Development of pediatric ulcerative colitis activity index (PUCAI). J Pediatr Gastroenterol Nutr 2006;43:E47).*

ITEM	POINTS
1. Abdominal pain:	
No pain	0
Pain can be ignored	5
Pain cannot be ignored	10
2. Rectal bleeding	
None	0
Small amount only, in less than 50% of stools	10
Small amount with most stools	20
Large amount (>50% of the stool content)	30
3. Stool consistency of most stools	
Formed	0
Partially formed	5
Completely unformed	10
4. Number of stools per 24 hours	
0-2	0
3-5	5
6-8	10
>8	15
5. Nocturnal stools (any episode causing wakening)	
No	0
Yes	10
6. Activity level	
No limitation of activity	0
Occasional limitation of activity	5
Severe restricted activity	10
SUM OF PUCAI (0-85)	

Figure 9: PUCAI score.

3. Extraintestinal Manifestations and Complications of Disease

The incidence of extraintestinal manifestations in IBD is estimated to be approximately 30% *(Oliva-Hemker M. More than a gut reaction:extraintesitnal complications of IBD. Contemp Pediatr 1999;16:45).* These complications often require immunomodulator and biologic therapy for their treatment.

Common extraintestinal manifestations of IBD in children are:

- Growth failure
- Sacroiliitis
- Osteopenia/osteoporosis
- Peripheral joint inflammation
- Aphtae
- Primary sclerosing cholangitis

- Granulomatous skin lesions, erythema nodosum, pyoderma gangrenosum
- Uveitis/episcleritis
- Ankylosing spondylitis

The pathogenesis of extraintestinal manifestations is unknown. One theory suggests that the uptake of bacterial products or dietary antigens can induce circulating immune complexes or a systemic inflammatory response. Another theory involves the cross-reaction with a bacterial epitope leading to autoimmunity directed against an antigen shared among the intestine, skin, synovium, eye and biliary system *(Bhagat S, Das KM. A shared and unique peptide in the human colon, eye, and joint detected by a monoclonal antibody. Gastroenterology 1994;107:103).* There is a strong genetic influence on extraintestinal manifestations: HLA-A2, -DR1 and DQw5 are more commonly found in Crohn disease patients with extraintestinal manifestations, while HLA-DRB1, -B27 and –B58 predispose patients with ulcerative colitis to these complications. HLA-B8/DR3 is associated with the development of other autoimmune conditions (celiac disease, autoimmune hepatitis, myasthenia gravis), and HLA B27 is reported in 50-80% of IBD patients with ankylosing spondylitis *(Rothfuss KS, et al. Extraintestinal manifestations and complications in inflammatory bowel disease. World J Gastroenterol 2006;12:4819).*

Growth failure occurs in 30% of children with CD and in 5-10% with UC. Some children can present delays in bone maturation and pubertal development. Proposed mechanisms are deficient nutrient intake, poor digestion and absorption, increased metabolic demands and adverse drug effects (i.e. corticosteroids). Cytokines may have a direct role in suppressing growth: TNF-α down-regulates growth hormone receptor formation *(Denson LA, et al. TNF-alpha downregulates murine hepatic growth hormone receptor expression by inhibiting Sp1 and Sp3 binding. J Clin Invest 2001;107:1451-1458)*, and synergistically with IL-1 increases chondrocyte death and reduces responsiveness to testosterone in animal models *(Martensson K, et al. Interleukin-1beta and TNF-alpha act in synergy to inhibit longitudinal growth in fetal rat metatarsal bones. J Bone Mineral Res 2004;19:1805-1812; Mauras N. Growth hormone in the glucocorticosteroid-dependent child: metabolic and linear growth effects. Horm Res 2001;56Suppl1:13-18).* The growth suppressive effects of corticosteroids include central suppression of GH release; decreased hepatic transcription of GH receptor; decreased insulin-like growth factor-1 (IGF-1) binding in cartilage *(Ballinger AB, et al. Delayed puberty associated with inflammatory bowel disease. Pediatr Res 2003;53:205-210).*

Inflammatory bowel disease occurring during early adolescence is likely to have a major impact on nutritional status and growth because of the rapid accumulation of lean body mass that normally

occurs at this time. Furthermore, boys are more prone to disturbances of growth than girls as their growth spurt occurs later and is ultimately longer and greater.

Prompt diagnosis of IBD and precise growth and pubertal evaluations are very important in avoiding growth retardation. Prevention and treatment strategies include avoidance of long and repeated steroid courses (better if using controlled release budesonide), more frequent use of enteral nutrition in Crohn disease, earlier consideration of surgical resection of localized Crohn disease and steoid-dependent ulcerative colitis. Anti-TNF-α agents may have an important role also in treating this compications, if treatment is undertaken early enough prior to or during puberty *(Griffiths AM, et al. Height of children with active Crohn disease improves during treatment with infliximab. Gastroenterology 2006;130 Suppl 2:A59).*

Joint manifestations are common: arthralgias are frequently reported, while arthritis occurs in up to 25% of patients. These can occur before or after the onset of IBD. Joint manifestations can be divided into an axial form (ankylosing spondylitis, sacroiliitis) and a peripheral form. The former is more common in ulcerative colitis and is treated with physical therapy, even if sometimes immunomodulators and biologics are needed. The peripheral form is more frequent in Crohn disease, is most typically associated to colonic inflammation and other extraintestinal manifestations; primary treatment of the bowel inflammation is the first course of action.

The prevalence of **osteoporosis** in adult population with IBD is 4-40%, while prevalence of osteopenia and osteoporosis in pediatric age is between 8 and 30% *(Gokhale R, et al. Bone mineral density assessment in children with inflammatory bowel disease. Gastroenterology 1998;114:902).* Pathogenic factors are inadequate intake or absorption of calcium and vitamin D, steroid use, low estrogen states in females, proinflammatory cytokines. Diagnosis is usually made with dual-energy x-ray absorptiometry (DEXA); peripheral quantitative computed tomography (pqCT) of the radius or tibia is currently being evaluated as a new diagnostic tool.

Bone modeling involves both bone-forming osteoblasts and bone-resorbing osteoclasts, with both cell types active at the same time on bone surfaces with net gain in bone mass. Bone remodeling, occurring in children and adults, is a slow process that involves the sequencial activities of osteoclasts and osteoblasts, so that resorption by osteoclasts precedes formation by osteoblasts. Bone modeling and remodeling may be affected by malnutrition, inflammation, inactivity, hypogonadism, and drugs such as corticosteroids *(Seeman E, Delmas PD. Bone quality . the material and structural basis of bone strength and fragility. N Engl J Med 2006;354:2250-2261).*

Osteoclasts are formed primarily by stimulation of precursor cells (monocyte lineage) with receptor activator of nuclear factor κB-ligand (RANKL) in the presence of macrophage stimulating

factor (M-CSF). RANKL is a member of the TNF receptor superfamily and is produced by osteoblasts, stromal cells and activated T-cells, and stimulates osteoclasts differentiation, activation and survival. Inflammatory cytokines such as TNF-α and IL-1β work via RANKL to increase osteoclast formation. Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL produced by osteoblasts and stromal cells, that works as an inhibitor of osteoclast development (besides the action of other cytokines such as IFN-γ, IL-10 and IL-12).

Osteoblasts have mesenchimal origin and are regulated by the interplay of several factors and hormones, such as IGF-1 and TNF-α; in human studies, anti-TNF-α therapy improves bone formation, and it is possible that this effect is obtained at least in part by a regulation of these pathways. Children with IBD commonly present decreased bone mineral density; it is not clear, however, if these patients are at increased risk of fractures.

Aphtous lesions occur more frequently among CD patients than UC patients. They tend to parallel intestinal disease.

Cutaneous lesions can be classified as granulomatous (ulcers, fistulas, subcutaneous nodules), reactive (erythema nodosum, pyoderma gangrenosum, Sweet's syndrome), and secondary to nutritional deficiencies (zinc, niacin, vitamin C). Erythema nodosum is the most frequent skin manifestation among pediatric patients; exacerbations often correlate with intestinal activity and show good response to infliximab *(Kugathasan S, et al. Dermatologic manifestations of Crohn disease in children: response to infliximab. J Pediatr Gastroenterol Nutr 2003;37:150-154).* Pyoderma gangrenosum is a rare, severe skin manifestation that consists in persisting, painful ulcers located on lower limbs; treatment is difficult, requiring steroids, immunomodulators, and infliximab in refractory cases.

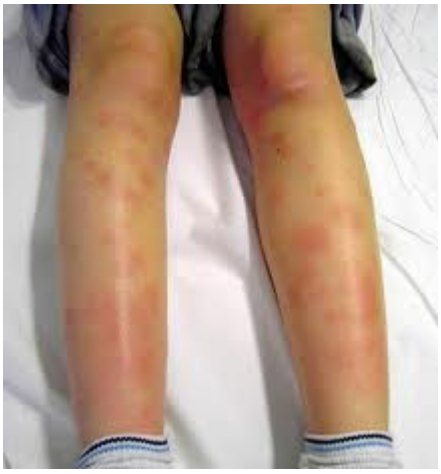

Figure 10: erythema nodosum.

Uveitis and episcleritis are often associated with other extraintestinal manifestations, especially arthritis and erythema nodosum. Episcleritis often parallels intestinal activity, while uveitis does not.

Liver manifestations are hepatitis, steatosis, cholelithiasis, amyloidosis and primary sclerosing cholangitis (PSC), and occur in up to 5% of patients. PSC is a chronic, fibrosing inflammation of unknown etiology affecting intra- and extrahepatic bile ducts leading to cirrosis, and is more frequently associated with ulcerative colitis. PSC can precede or follow the diagnosis of IBD, especially in children. Treatment options are choleretic agents such as ursodeoxycholic acid and, in advanced stages, liver transplantation.

Other less frequent extraintestinal manifestations can affect hemopoietic system, vascular system, pancreas, renal system, central and peripheral nervous system, heart.

4.1 Medical therapy of IBD

The goals of therapy are to induce and maintain clinical and endoscopic remission (mucosal healing). Additional therapeutic goals in children are to restore growth and promote development; these need to be achieved within a relatively short window of opportunity, before growth retardation and developmental deficiencies become permanent.

Medications used for IBD therapy are: 5-aminosalicylates, antibiotics and probiotics, corticosteroids, 6-mercaptopurine and its metabolite azathioprine, other immunosuppressors such as methotrexate, cyclosporine, tacrolimus, and biologic therapies, mostly anti-TNF-α monoclonal antibodies such as infliximab and adalimumab. In children, enteral feeding with monomeric or polymeric formulae can be used to achieve and maintain remission and to promote growth.

Aminosalicylates are used in mild to moderate IBD, particularly in ulcerative colitis *(Bergman R, Parkes M. Systematic review: the use of mesalazine in inflammatory bowel disease. Aliment Pharmacol Ther 12006;23:841-855).* Sulfasalazine is a combination of sulfapyridine, a sulfa antibiotic, and 5-aminosalicylic acid (5-ASA), which has anti-inflammatory properties. Breaking of the azo bond by colonic bacteria releases 5-ASA and sulfapyridine, which is then absorbed and is mainly responsible of the anti-rheumatic as well as the adverse effects. Currently, sulfasalazine is rarely used (i.e. for patients with extraintestinal joint disease), and several formulations of 5-ASA are available to target the release of the drugs in specific sites of the gastrointestinal tract. Topical formalations are also available, in the form of suppositories and enemas, for the treatment of distal ulcerative colitis.

Mechanisms of action are inhibition of cyclo-oxygenase and 5-lipoxygenase, anti-oxidant and free radical scavenging, and immunomodulating actions. *(MacDermott RP. Progress in understanding the mechanism of action of 5-aminosalicylic acid. Am J Gastroenterol 2000;95:3343-3345).* There is increasing evidence that long-term 5-ASA therapy can significantly decrease the risk of colon cancer in patients with ulcerative colitis, the effect being more pronounced with higher doses *(Velayos FS, et al. Efect of 5-aminosalycilate use on colorectal cancer and dysplasia risk: a systematic review and meta-analysis of observational studies. Am J Gastroenterol 2005;100:1345-1353).* The most common adverse effects are headache, diarrhea, abdominal pain, and nausea; rarely fever, rash, agranulocytosis, hemolysis, oligospermia, nephritis, alveolitis have been reported.

Antibiotics possess the ability of change the course of IBD in a variety of ways, including reducing luminal bacterial content, changing the colonic flora, reducing bacterial invasion of intestinal wall and limiting bacterial translocation *(Perencevich M, Burakoff R. Use of antibiotics in the treatment of inflammaroty bowel disease. Inflamm Bowel Dis 2006;12:651-664).* The most used antibiotics are rifaximin, metronidazole and ciprofloxacin. Indications for antibiotic use are active Crohn disease, perianal disease (fistula, abscess), post-operative recurrence prevention, ulcerative colitis exacerbations, and pouchitis *(Campieri M, et al. Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn disease: a randomized controlled study versus mesalazine. Gastroenterology 2000;118(Suppl1):A781 – Gionchetti P et al. Antobiotic combination therapy in patients with chronic, treatment-resistant pouchitis. Aliment Pharmacol Ther 1999;13:713-718).*

Nutritional therapy is useful in the treatment of children with IBD who often present nutritional deficits, bone disease, weight loss, linear growth impairment and pubertal delay. Polymeric formulae are preferred over elemental ones because of their higher palatability in face of similar efficacy. In children, it seems that nutritional therapy and steroid therapy are equally effective in inducing remission *(Heuschkel RB, et al. Enteral nutrition and corticosteroids in the treatment of acute Crohn disease in children. J Pediatr Gastroenterl Nutr 2000;31:8-15),* particularly in newly diagnosed Crohn disease with small bowel involvement; suggested treatment duration with exclusive formula feeding is eight weeks *(Day AS, et al. Exclusive enteral feeding as primary therapy for Crohn disease in Australian children and adolescents: a feasible and effective approach. J Gastroenterol Hepatol 2006;21:1609-1614).* After the successful induction of remission by enteral nutrition in active CD, ongoing use as a maintenance therapy may be achieved by regular overnight nasogastric feeds, by oral supplements in addition to usual diet, or by intermittent cycles alternating with normal diet *(Takagi S, et al. Effectiveness of an "half elemental*

diet" as maintenance therapy for Crohn disease. A randomized controlled trial. Aliment Pharmacol Ther 2006;24:1333-1340). Mechanisms of action include direct anti-inflammatory effects and alteration of intestinal microflora *(Lionetti P, et al. Enteral nutrition and microflora in pediatric Crohn disease. J Parenter Enteral Nutr 2005;29(Suppl 4):S173-175; discussion S 175-178, S184-188).*

Probiotics are live microorganisms that confer a health benefit when administered in adequate amounts. Most microorganisms are bacteria that have been derived from human, animal and food sources. Most species of probiotics belong to the Lactobacillus and Bifidobacterium genus; another frequently administered one is the fungus Saccharomyces boulardii. Probiotics have been evaluated for the treatment of active ulcerative colitis showing benefit; when used for maintenance therapy in UC, probiotics had similar effect compared to mesalazine *(Kato K, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. Aliment Pharmacol Ther 2004;20:1133-1141; Venturi A, et al. Impact onthe composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 1999;13:1103-1108).* Pouchitis is the most common long-term complication following ileal pouch-anal anastomosis for acute ulcerative colitis. Most patients develop this problem in their first year following the procedure and luminal flora has been shown to play a prominent role in the pathogenesis of pouchitis. It has been shown that probiotics are more useful in preventing initial post-operative onset of pouchitis than treating it once it occurs *(Gionchetti P, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 2003;124:1202-1209).* In Crohn disease, probiotics do not seem to be effective neither for maintenance therapy nor for prevention of post-operative recurrence *(Bousvaros A, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm Bowel Dis 2005;11:833-839).* Side effects are rare and consist in worsening of diarrhea and abdominal pain; however, in severely immunocompromised patients, probiotics have the potential to induce sepsis *(Munoz P, et al. Saccharomyces cerevisiae fungemia: an emerging infectious disease. Clin Infect Dis 2005;40:1625-1634).*

Glucocorticoids have been used for decades as first-line treatment to induce remission in pediatric and adult IBD. Upon binding if the glucocorticoid receptor, a cascade of events take place starting with the dissociation of molecular chaperones followed by nuclear translocation. Specific DNA sequences of steroid-responsive genes are bound, leading to suppression of the genes encoding for the transcription of inflammatory proteins (e.g. mitogen-activated protein kinase – MAPK pathway). Nongenomic mechanisms may also be involved, such as the activation of

endothelial nitric oxide synthase. Corticosteroids can be administered systemically or topically. Steroids do not heal mucosa in IBD *(Travis SP, et al. European evidence based consenss on the diagnosis and management of Crohn disease: current management. Gut 2006;55 (Suppl 1):16-35);* steroid-dependency at one year after diagnosis occurs in 31% of children with CD and in 45% with UC *(Markowitz J, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn disease. Clin Gastroenterol Hepatol 2006;4:1124-1129; Hyams J, et al. The natural history of corticosteroid therapy for ulcerative colitis in children. Clin Gastroenterol Hepatol 2006;4:1118-1123).* Side effects include adrenal insufficiency, osteoporosis, cosmetic effects, cataract, glaucoma, hypertension, glucose intolerance, psychiatric effects. Budesonide, a glucocorticosteroid with a weak mineralocorticoid activity, can be released in the terminal ileum and/or in the colon and undergoes an extensive first-pass hepatic degradation to metabolites with a low glucocorticosteroid activity. It is useful in inducing remission in mild to moderate ileocecal CD. Side effects are significantly less common with budesonide than with prednisolone; the most frequent are adrenal suppression, headache, growth impairment. Topical steroid treatment is useful in distal ulcerative colitis. The current trend in pediatric and adult Crohn disease is to minimize and avoid repeated courses of steroids by introducing immunomodulators early in the course of disease.

6-mercaptopurine (6-MP) and its prodrug **azathioprine** (AZA) are the immunosuppressant drugs of choice for patients with steroid dependent IBD, allowing corticosteroid withdrawal in 40-70% of patients *(Pearson DC, et al. Azathioprine and 6-mercaptopurine in Crohn's disease: a meta-analysis. Ann Intern Med 1995;122:132-142).* This drugs suppress disease activity in up to 70% of children with IBD, and among these patients 50% will achieve a clinical response after 4 months of continuous therapy *(Verhave M, et al. Azathioprine in the treatment of children with inflammatory bowel disease. J Pediatr 1990;117:809-814).* AZA/6-MP can reduce the re-operation rate in patients who underwent intestinal resection and ileo-colonic anastomosis if compared to mesalazine and placebo *(Hanauer SB, et al. Post-operative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine or placebo: a 2 year trial. Gastroenterology 2004;127:723-729).* AZA and 6-MP have to be metabolized into their active forms to interfere with ribonucleotide replication, inducing lymphocytotoxicity and immunosuppression *(Carvalho RS, et al. Inherent resistance to 6-thioguanine induced apoptosis correlates with disease activity in children with IBD. Gastroenterology 2006;A203).* The principal catalytic enzyme is TPMT (thiopurine S-methyltransferase). TPMT enzyme activity and consequently efficacy and side effects are influenced by genetic polymorphism, and it is possible to perform TPMT phenotype testing as well as metabolite monitoring to evaluate efficacy and to find patients at risk for severe bone

marrow suppression *(Evans WE, et al. Altered mercaptopurine metabolism, toxic effects, and dosage requirements in a thiopurine methyltransferase deficient child with acute lymphoblastic leukemia. J Pediatr 1991;119:985-989).* Other adverse effects are pancreatitis, hepatitis and malignancies including acute myeloblastic leukemia, myelodisplasia and hepatosplenic T-cell lymphoma (in patients on concomitant anti-TNFα therapy) *(Thayu M, et al. Hepatosplenic T-cell lymphoma in an adolescent patient after immunomodulator and biologic therapy for Crohn disease. J Pediatr Gastroenterol Nutr 2005;2:220-222).*

Methotrexate (MTX) is an alternative immunosuppressor to AZA/6-MP. It is a competitive antagonist of folic acid thus inhibiting dihydrofolate reductase which is critical to purine and pyrimidine synthesis; MTX blocks DNA production and exerts anti-proliferative as well as cytotoxic effects when administered at higher doses *(Chabner BA et al. Antineoplastic agents. In: Hardman JG et al Editors. Goodman and Gilman's the pharmacological basis of therapeutics. 9th Edition. New York: McGraw-Hill 1996;1243-1247).* In Crohn disease patients MTX is used at low doses, and the precise mechanism of action is still not known but possibly involves T-cell apoptosis and reduction of cytokine secretion among others *(van Dieren JM, et al. Revisiting the immunomodulators tacrolimus, methotrexate and mycophenolate mofetil: their mechanisms of action and role in the treatment of IBD. Inflamm Bowel Dis 2006;12:311-327).* MTX has been found to be effective in adult IBD *(Ardizzone S, et al. Comparison between methotrexate and azathioprine in the treatment of chronic active Crohn disease: a randomized, investigator-blind study. Dig Liver Dis 2003;35:619-627);* experience in pediatric IBD is scant, however it is commonly used for juvenile idiopathic arthritis *(Woo P, et al. Radomize, placebo-controlled, crossover trial of low dose oral methotrexate in children with extended oligoarticular or systemic arthritis. Arthritis Rheum 2000;43:1849-1857).* Side effects are usually transient and respond to dose reduction: nausea, abdominal pain, stomatitis, bone marrow suppression, hepatotoxicity, infections, skin rash; teratogenity makes it contraindicated in pregnancy.

Infliximab (IFX) is a chimeric monoclonal IgG1 antibody to TNF-α composed by a 75% human constant and a 25% murine variable region. Besides TNF-α neutralisation, IFX also blocks leukocytes migration and induces apoptosis of T-lymphocytes and monocytes *(Van den Brande JM, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn disease. Gastroenterology 2003;124:1774-1785).* A third mechanism involves complement fixation and complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity *(Scallon BJ, et al. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. Cytokine 1995;7:251-259).* In adult patients with Crohn disease, infliximab has been found to be effective in:

inducing and maintaining remission (response in 80% after one infusion and in 48% after one year of treatment in the ACCENT 1 trial); discontinuing steroids in virtually all corticosteroid-dependent patients; achieving mucosal healing in 50% at one year *(Rutgeers P, et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn disease. Gastrointest Endosc 2006;63:433-442);* closing perianal fistulas in 55% of the patients *(Present DH, et al. Infliximab for the treatment of fistulas in patients with Crohn disease. N Engl J Med 1999;340:1398-1405).* In pediatric Crohn disease, the REACH study showed remission at 54 weeks in 59% of patients and response in 88% *(Hyams J, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863-873).* A number of studies reported beneficial effects of IFX in restoring growth and height as early as 6 months after the start of therapy *(Griffiths AM, et al. Height of growth delayed children with active Crohn disease improves during treatment with infliximab. Gastroenterology 2006;130:A12)* and more prominently in patients on systematic re-treatment. Studies confirmed the steroid-sparing effect of IFX in 46-67% of patients *(Stephens MC, et al. Safety and steroid-sparing experience using infliximab for Crohn disease at a pediatric inflammatory bowel disease center. Am J Gastroenterol 2003;98:104-111 . Lamireau T, et al. Efficacy and tolerance of infliximab in children and adolescents with Crohn disease. Inflamm Bowel Dis 2004;10:745-750).* As in adults, systematic maintenance therapy with IFX infusions scheduled every 8 weeks is superior to episodic therapy or even maintenance therapy every 12 weeks in maintaining response and remission.

There is evidence that more aggressive therapy early in CD is associated with better response and remission rates *(Lionetti P, et al. Response to infliximab is related to disease diration in pediatric Crohn disease. Aliment Pharmacol Ther 2003;18:425-431),* also with infliximab and concomitant immunosuppressors in adult and pediatric patients *(Vermeire S, et al. Demographical and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn disease. Am J Gastroenterol 2002;97:235-2363 – Hommes D, et al. A randomized controlled trial evaluating the ideal medical management for Crohn disease: top-down versus step-up strategies. Gastroenterology 2005;128:A-577).* The cumulative incidence of surgery among pediatric Crohn disease patients is 17% at 5 years and 28% at 10 years; the treatment with infliximab in a cohort of 989 pediatric patients was associated with a 64% reduced risk for surgery *(Gupta N, et al. Risk factors for initial surgery in pediatric patients with Crohn disease. Gastroenterology 2006;130:1069-1077).*

In adult UC, the efficacy of infliximab administered 5 mg/kg every 8 weeks has been demonstrated by the ACT-1 and ACT-2 trials *(Rutgeers P, et al. Infliximab for induction and*

maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-2476). IFX is also useful as a rescue therapy to avoid colectomy in fulminant colitis, but long-term data are still lacking. Pediatric studies indicate 75% of complete short-term response and 25% of colectomy rate at 10 months follow-up, with a better response in those on concomitant 6-MP treatment *(Eidelwein AP, et al. Infliximab efficacy in pediatric ulcerative colitis. Inflamm Bowel Dis 2005;11:213-218).*

One of the most common problems of infliximab includes its chimeric nature and the formation of antibodies to the murine portion of the drug (ATI). These ATIs are neutralizing and interfere with the safety and efficacy of the drug, are associated with acute infusion reactions and loss of response with delayed hypersensitivity phenomena. Acute infusion reactions are shortness of breath, chest pain, palpitations, flushing, headache, urticaria and hypotension. The prevalence of acute infusion reactions ranges between 8 and 25% *(Friesen CA, et al. Safety of infliximab treatment in pediatric patients with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2004;39:265-269 , Colombel JF, et al. The safety profile of infliximab in patients with Crohn disease: the Mayo clinic experience in 500 patients. Gastroenterology 2004;126:19-31),* while anaphylaxis is rare. Acute infusion reactions are controlled by slowing down or temporarily stopping the infusion and administering anti-histamines or hydrocortisone acutely as well as in subsequent infusions. Delayed infusion reactions occur after 4-9 days after an infusion, and are characterized by arthralgias, back pain, myalgias, fever, skin rash and leukocytosis; these manifestations are rare but need oral corticosteroids or the switch to humanized anti-TNF. The formation of ATI may be suppressed by concomitant therapy with immunosuppresants, with pretreatment with hydrocortisone and with systematic infusions as opposed to episodic therapy *(Rutgeers P, et al. Comparison of scheduled and episodic treatment strategies of infliximab in Crohn disease. Gastroenterology 2004;126:402-413).* Another immunologic phenomenon associated with anti-TNF use is the formation of anti-nuclear antibodies (ANA) and antibodies to double stranded DNA (anti-dsDNA) *(Vermeire S, et al., Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn disease: a prospective cohort study. Gastroenterology 2003;125:32-39),* the first occurring in 50% of patients, the latter in 25%. A rare consequence of this phenomenon is the development of drug-induced lupus.

There is an increased risk of reactivation of latent tuberculosis associated with anti-TNF therapy; therefore, all patients undergoing this kind of therapies should be screened for latent tuberculosis. Respiratory tract infections are more common in patients on infliximab, but these are usually mild and respond to supportive therapy and antibiotics. The risk for serious infections is more frequent in patients receiving IFX and concomitant steroids or immunomodulators *(Toruner M, et al. Risk factors for opportunistic infections in inflammatory bowel disease: a case-control study.*

Gastroenterology Suppl 2006;A-71); therefore, corticosteroids should be tapered and stopped as quickly as possible, and azathioprine/6-MP can be stopped safely after an initial period of 6 months, when maintenance therapy with IFX is continued *(Van Assche G, et al. Continuation of immunomodulators is not required to maintain adequate infliximab efficacy in patients with Crohn disease but may improve pharmacokinetics. Gastroenterol Suppl 2006;130:A-142).*

A safety meta-analysis showed an increased risk for malignancies in patients treated with IFX (with a dose ≥6 mg/kg) for rheumatoid arthritis, with an Odds ratio of 4.3 (95% CI 1.6-11.8) *(Bongartz T, et al. Anti-TNF-alpha antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. JAMA 2006;295:2275-2285).* There are reports of 18 cases of hepatosplenic T-cell lymphoma in patients with Crohn disease treated with IFX and AZA/6-MP, including pediatric patients *(Rosh JR, et al. Hepatosplenic T-cell lymphoma in adolescents and young adults with Crohn's disease: a cautionary tale? Inflamm Bowel Dis 2007;13:1024-1030);* therefore, a special concern is the concomitant immunosuppressive therapy, which should be carefully evaluated for these patients *(Harrington LJ, et al. Role of thiopurine and anti-TNF therapy in lymphoma in inflammatory bowel disease. Am J Gastroenterol 2011;106:2146-2153).*

Other anti-TNF-α agents that have been evaluated for the treatment of Crohn disease include adalimumab, certolizumab pegol, etanercept and onercept.

Adalimumab (ADA) is a fully human IgG1 monoclonal antibody to TNF-α that fixes complement, mediates antibody-dependent cytotoxicity and induces T cell apoptosis *(Shen C, et al. Capase activation and apoptosis induction b adalimumab: demonstration in vitro and in vivo in a chimeric mouse model. Inflamm Bowel Dis 2006;12:22-28).* It is administered subcutaneously and has a half-life of 12-14 days. Controlled trials have shown that ADA is effective for the treatment of rheumatoid arthritis , psoriatic arthritis, ankylosing spondylitis. In the CLASSIC 1 and 2 trials, patients with Crohn disease naïve to anti-TNF agents received ADA induction and maintenance: the optimal inducing regimen was 160 mg at week 0 and 80 at week 2, with a remission rate of 36% at week 4. Patients in remission at week 4 were randomized to receive 40 mg every other week (showing a 69% remission rate) or 40 mg weekly (with a remission rate of 83%) *(Sandborn WJ, et al. Adalimumab for maintenance treatment of Crohn disease: results of the CLASSIC II trial. Gut 2007;56:1232-1239).* Patients that did not maintain remission at week 4 received open-label adalimumab 40 mg every other week, with escalation to weekly doses of 40 mg for flare or non-response; at week 56 the rates of response and remission were 69% and 44%. The CHARM trial examined maintenance of remission in patients who responded to induction therapy with adalimumab, including those who had previously been treated with infliximab: at week 4, 58% of

patients achieved response; at week 56, the rates of response with placebo, ADA 40 mg every other week, ADA 40 mg weekly were 18%, 43% and 47% respectively; at week 56, the rates of remission with placebo, ADA 40 mg every other week, ADA 40 mg weekly were 12%, 36% and 41% respectively *(Colombel JF, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn disease: the CHARM trial. Gastroenterology 2007;132:56-65).* There was no statistical significance between the rates of response and remission in the groups randomized to receive ADA weekly or every other week, and neither prior therapy with infliximab nor concomitant immunosuppressor therapy influenced the results. Of 117 patients with fistulas, complete healing was achieved by 33% of those on adalimumab, compared to 13% who received placebo; adalimumab was also steroid-sparing . Other studies showed that patients who had intolerance or prior loss of response to infliximab could respond to adalimumab *(Papadakis KA, et al. Safety and efficacy of adalimumab (D2E7) in Crohn disease patients with attenuated response to infliximab. Am J Gastro 2005;100:75-79)*: in the GAIN trial, patients with moderate to severe Crohn disease who had previously responded to infliximab and then lost response or became intolerant were randomized to receive adalimumab 160 mg at week 0 and 80 mg at week 2 or placebo. At week 4, the rates of response in the placebo and adalimumab groups were 34% and 52%; the rates of remission were 7% and 21% respectively *(Sandborn WJ, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab; a randomized trial. Ann Intern Med 2007;146:829-838).* Some pediatric studies found adalimumab to be useful in Crohn disease *(Mian S, Baron H. Adalimumab, a novel anti-tumor necrosis factor-alpha antibody in a child with refractory Crohn disease. J Pediatr Gastroenterol Nutr 2005;41:357-359).*

The most common reported adverse effects with adalimumab are localized injection reactions. In patients with rheumatoid arthritis treated with ADA, drug-induced lupus, demyelination, lymphoma and serious and opportunistic infections have been reported *(Prescribing information for Humira (adalimumab), 2007).* In post-marketing surveillance, the rate of serious infections in adalimumab-treated patients was 4.1 per 100 patient-years; the rate of demyelinating disorders was 0.08 per 100 patient-years. Analysis of the incidence of lymphoma in adalimumab clinical trials, in comparison to the normal population, resulted in a SIR of 3.19 (95% CI 1.78 to 5.26); the types of lymphomas include Hodgkin's disease, B cell lymphoma, T-cell lymphoma, central nervous system lymphoma, and mucosa associated lymphoid tissue lymphoma (MALT) *(Schiff MH, et al. Safety analyses of adalimumab (HUMIRA) in global clinical trials and IS postmarketing surveillance of patients with rheumatoid arthritis. Ann Rheum Dis 2006;65:889.894).*

Certolizumab pegol showed some efficacy in controlled trials performed in adults *(Sandborn WJ, et al. Certolizumab pegol for the treatment of Crohn disease. N Engl J Med 2007;357:228.238).*

Other biologics with limited data are CDP571, onercept, natalizumab and MLN02 (monoclonal antibodies against the adhesion molecule α4β7 integrin), alicaforsen (anti-ICAM1), daclizumab and basiliximab (anti-IL2 receptor), visilizumab (anti-CD3), among others.

4.2 Surgical therapy

70-80% of patients with Crohn disease undergo some type of surgical procedure during the course of their disease *(Poggioli G, et al. Review article: indication and type of surgery in Crohn's disease. Alim Pharmacol Ther 2002;16 Suppl 4:59-64).* The most frequent indications are obstruction, perforation, medical treatment failure, fistula, abscess, bleeding. In as many as 50% of pediatric patients the indication of surgery may be failure of medical therapy with growth retardation rather than obstruction or other mechanical complications *(Patel HI, et al. Surgery for Crohn's disease in infants and children. J Pediatr Surg 1997;32:1063-1067).* The timing of surgery for growth issues is critical in the adolescent, because surgery must occurr before epiphyseal plates close to allow catch up growth following the procedure *(Konno M, et al. Guidelines for the treatment of Crohn's disease in children. Pediatr Int 1006;48:349-352).* Surgical emergencies in Crohn disease are perforation with peritonitis or obstruction. Elective surgery indications are failure of medical management, strictures, fistulas.

Perianal fistulae may be simple (below the dentate line, superficial origin, external opening); or complex (high intersphinteric, transsphinteric or supraphinteric origin, multiple external openings, painful and associated with rectovaginal fistula anorectal stricture). Perianal fistulae may develop locally as deep penetrating ulcers in the anus or rectum, or alternatively after anal gland infections or abscesses *(Hughes L. Surgical pathology and management of anorectal Crohn disease J R Soc Med 1978;71:644-651).* The reported incidence of fistulas in CD patients ranges from 17% to 43% *(Van Dongen LM, Lubbers E. Perianal fistulas in patients with Crohn disease. Arch Surg 1986;121:1187-1190);* most of them are perianal, followed by enteroenteric, rectovaginal, enterocutaneous, enterovescical and entero-intraabdominal. Simple fistulae may heal spontaneously in up to 50% of cases, whereas complex fistulae rarely do so *(Judge TA, Lichtenstein GR. Treatment of fistulizing Crohn disease. Gastroenterol Clin N Am 2004;33:421-454).* Perianal disease can be studied performing MRI, endoscopic ultrasound and examination under anesthesia (EUA); the most accurate results are obtained when any two out of the three techniques are used *(Schwartz DA, et al. A comparison of endoscopic ultrasound, magnetic resonance imaging, end exam under anesthesia for evaluation of Crohn perianal fistulas. Gastroenterology 2001;121:1064-*

1072). Treatment of perianal fistulae consists of antibiotics, immunomodulators (AZA/6-MP), biologics (infliximab, adalimumab). A meta-analysis of studies evaluating AZA/6-MP reported an overall response rate (defined as improvement or complete healing) in 54% of patients compared to 21% in patients treated with placebo *(Korelitz BI, Present DH. Favorable effect of mercaptopurine on fistulae of Crohn disease. Dig Dis Sci 1985;30:58-64);* the corresponding pooled odds ratio for fistula healing with AZA/6-MP was 4.44 (95% CI 1.50 – 13.20). Other studies showed similar results and underscored the importance of maintenance therapy *(Jeshion WC, et al. Azathioprine and 6-mercaptopurine for the treatment of perianal disease in children. J Clin Gastroenterol 2000;30:294-298).* Fistulae remain closed for 1-5 in 46% of patients, who remained on 6-MP, and relapses tended to occur within 2 weeks to 9 months after discontinuation of the drug. Infliximab has been shown to be efficacious *(Sands BE, et al. Infliximab maintenance therapy for fistulizing Crohn disease. N Engl J Med 2004;350:876-885):* IFX 5 mg/kg at least halved the number of draining fistulae in 68% of patients compared to 26% of patients who received placebo; closure of all fistulae was achieved in 55% of patients receiving IFX compared to 13% of patients receiving placebo. The median time to response was 14 days for IFX-treated patients versus 42 days for patients assigned to placebo; the median duration of response was three months, suggesting that maintaining therapy may be required. The ACCENT II trial confirmed the response rate of the previous study and pointed out that the median time to loss of response was 40 weeks in infliximab-treated patients and 14 weeks in placebo-assigned patients. Overall, 42% of patients in the infliximab group and 62% of the placebo group experienced loss of response *(Sands BE, et al. Infliximab maintenance therapy for fistulizing Crohn disease. N Engl J Med 2004;350:876-885).* According to one study, ciprofloxacin may add benefit when concomitant therapy with infliximab is performed *(West RL, et al. Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulae in Crohn disease with infliximab: a double-blind placebo-controlled study. Aliment Pharmacol Ther 2004;20:1329-1336).* Uncontrolled studies reported that combined infliximab and AZA/6MP or methotrexate adds benefit versus infliximab therapy alone *(Schroder O, et al. Combining infliximab and methotrexate in fistulizing Crohn disease resistant or intolerant to azathioprine. Aliment Pharmacol Ther 2004;19:295-301).* The combination of infliximab with surgical intervention (EUA with seton placement) was more effective compared to infliximab therapy alone in achieving and maintaining response for a longer period of time *(Van der Hagen SJ, et al. Anti-TNF-alpha (infliximab) used as induction treatment in case of active proctitis in a multistep strategy followed by definitive surgery of complex anal fistulas in Crohn disease: a preliminary report. Dis Colon Rectum 2005;48:758-767).* Adalimumab was found helpful in fistulizing Crohn disease, with a response rate of 56% at week 12 and a complete closure at week 4

in 33% of patients *(Sandborn WJ, et al. An open-label study of the human anti-TNF monoclonal antibody adalimumab in subjects with prior loss of response or intolerance to infliximab for Crohn disease. Am J Gasstroenterol 2004;99:1984-1989).*

30-40% of patients with ulcerative colitis require surgery at some point of the disease *(Hancock K, et al. Inflammatory bowel disease: the view of the surgeon. Colorectal disease 2006;8 Suppl 1:10-14).* Indications are failure of medical therapy, complications (bleeding, sepsis, perforation, stricture), mucosal dysplasia with risk of malignancy. The current standard of care is proctocolectomy with ileal pouch-anal anastomosis (IPAA), the most commonly used pouch configuration being the "J" pouch. Most surgeons perform a temporary ileostomy to reduce the risks of pelvic abscess and other complications. Functional results are usually very good. Most complications occur in the immediate postoperative period and are easy to manage: infections, ileus, parastomal hernial, pelvic abscess, strictur of the ileo-anal anastomosis. Pouchitis occurs in 15-53% of patients; the diagnosis is based on clinical symptoms and endoscopic and hystologic findings *(Penna C, et al. Pouchitis after ileal pouch-anal anstomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. Gut 1996;38:234-239).* Pouchitis can be acute or chronic; a pouchitis disease activity index has been developed and is based on clinical, endoscopic and histologic findings. Most patients respond to antibiotics, probiotics, some require immunomodulators and in 5% of cases removal of the pouch and permanent ileostomy *(Kooros K, Katz AJ. Infliximab therapy in pediatric Crohn pouchitis. Inflamm Bowel Dis 2004;10:417-420 , Shen B, et al. Maintenance therpay with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. Alim Pharm Ther 2005;11:318-325).* Women who have undergone IPAA may develop infertility *(Gorgun E, et al. Fertility is reduced after restorative proctocolectomy with ileal pouch anal anastomosis: a study of 300 patients. Surgery 2004;136:795-803).*

Part II: Investigation

Patients and methods

We aimed to evaluate the role of anti-TNF-alpha therapy with infliximab and adalimumab in a cohort of pediatric patients followed by our Center from 2002 to 2012.

Our Unit takes care of 50 pediatric patients out of 5500 total IBD patients; 21 are females and 29 males, with a diagnosis of IBD established before 18 years of age: 41 with Crohn disease, 8 with ulcerative colitis (one of which also has celiac disease), 1 with pouchitis after an initial diagnosis of ulcerative colitis. Seven patients were not considered for the analysis due to incomplete data. Forty patients out of forty-three were treated with anti-TNF-α biologic agents, while three patients received only 5-ASA, antibiotics, or steroids, and were not considered for the analysis.

The diagnosis was made according to established endoscopic, radiologic and histopathologic criteria.

Medical records were reviewed for the following informations: age, sex, age at diagnosis, disease location and behavior, laboratory results, medical therapy, particularly treatment with steroids (number of courses), immunosuppressants (type and age at onset), infliximab (indications, time and number of infusions, efficacy, side effects) and adalimumab (indications, duration of therapy, efficacy, side effects), surgical therapy (indications, number and type of procedures). Primary non response to infliximab was defined as absence of clinical and/or endoscopic response or worsening of symptoms at least 4 weeks after 2 infusions; loss of response to IFX was defined as initial response followed by reduction in efficacy, requiring a shortening of the interval between infusions to < 6 weeks and/or increase of the dose to 10 mg/kg, and eventual loss of response.

Loss of response to adalimumab was defined as return of previous symptoms or development of new complaints during adalimumab treatment after a period of partial or complete improvement.

Patients were screened for HBV (HBsAg and antibodies) and HCV (antibodies), for tuberculosis using a protein-purified derivative skin test and chest radiograph before the initiation of anti-TNF treatment.

Statistical analysis for continuous variables is given as median with range. The results for non-continuous variables are given as frequencies and percentage. Microsoft Excel 2010 and SPSS (version 14.0) were used to perform statistics.

Results

The cohort of patients examined consisted of 40 patients: 34 with Crohn disease (85%), 5 with ulcerative colitis (12.5%), one with chronic pouchitis after IPAA for ulcerative colitis (2.5%).

All patients were treated with the anti-TNF-α biologic agents infliximab and adalimumab.

Thirty-six received infliximab therapy: 19/36 received only infliximab, 17/36 received infliximab and then adalimumab due to loss of response to infliximab and steroid dependency; 4 patients received only adalimumab (infliximab-naïve).

Anti-TNF treatment was started before 18 years of age in 34 patients: 29 received infliximab and 5 started adalimumab during childhood.

Infliximab therapy

Thirty-six patients were treated with infliximab, 21 males, 15 females. Twenty-nine out of 36 had Crohn disease, 6 ulcerative colitis, and 1 pouchitis.

Median age at diagnosis was 15 years (range 10-18 years, mean 14.4 years). Median disease duration was 1.4 years (mean 2 years, range 0.2-11 years).

According to the Montreal Classification, Crohn disease was ileal (L1) in 3 patients, colonic (L2) in 5 patients, ileocolic (L3) in 19 patients, ileocolic and upper GI (L3+L4) in 2 patients; there was no isolated upper GI (L4) disease. Behavior type was inflammatory in 15 patients, stricturing in 3 patients, fistulizing in 11 patients.

Ulcerative colitis had the features of a pancolitis in 6 patients, while involved the discending colon in 1 patient. The patient with pouchitis underwent a colectomy with ileal pouch-anal anastomosis for ulcerative pancolitis, and later developed a perianal fistula.

Extraintestinal manifestations were recorded in 8 patients (22.2%): 3 dermatological (2 erythema nodosum, 1 pyoderma gangrenosum), 5 to the skeletal system (2 osteoporosis, 2 arthralgias, 1 sacroiliitis).

Fourteen patients (38.9%) had perianal disease: 12 had CD, 1 had UC (during the second course of IFX while he was also taking oral steroids), 1 had pouchitis.

Previous therapies consisted of steroids and immunosuppressive agents. Twenty-one patients (58.3%) received systemic corticosteroids, with a mean number of 2.4 courses per patient; 25

patients received immunosuppressants: 24 Azathioprine/6-mercaptopurine, 2 methotrexate (one previously received AZA), and median age at the beginning of drug treatment was 15.5 years.

Concomitant medications (other than mesalazine, topical steroids or antibiotics) were azathioprine/6MP (10 patients) and systemic steroids (4 patients).

Patients received IFX 5 mg/kg at 0, 2 and 6 weeks (induction), and then maintenance therapy 5 mg/kg every 8 weeks.

Mean age at first dose was 16.4 years (median 16.4, range 12.2-24), mean number of infusions 7.2 per patient (median 6, range 2-19), mean duration of therapy 9.8 months (median 8, range 1-29). Indications for biologic use were: steroid-dependency (14 patients) or chronically active disease resistant to other therapies (22 patients).

The median duration of follow-up after the onset of biologic therapy was 31.5 months (mean 38.5 months, range 1-116 months).

Overall, 13/36 patients (36.1%) reported complete remission of symptoms lasting for 19.8 months (mean; range 6-48) after IFX start; 20/36 patients (55.6%) reported improvement of symptoms for a mean duration of 16.2 months (mean; range 2-60) after IFX start; 3/36 patients (8.3%) did not experience any improvement.

Endoscopy was available for 15/36 patients: in 4 of them (26.7%) there was mucosal healing lasting for a mean period of 17.5 months (range 10-24) from IFX start; for 8 patients (53.3%) there was improvement but not endoscopic remission lasting for a mean period of 12.2 months (range 3-21) from IFX start; in 5 patients (33.3%) there was no endoscopic improvement.

Laboratory response was evaluated in 13/36 patients: C-reactive protein normalization (levels < 0.6 mg/dl) was evident in 4/13 patients (30.7%), and lasted for a mean duration of 19.8 months (range 6-32) after IFX start; in 4/13 patients (30.7%) there was a reduction but not normalization of CRP levels; 5/13 patients (38.5%) did not show CRP reduction.

Eight adverse events over 254 infusions (3.1%) were recorded: 5 infusion reactions (skin rash, shortness of breath, nausea) controlled by temporarily stopping infusion and then restarting it at a slower rate, 1 hypersensitivity allergic reaction leading to interruption of IFX treatment, 1 mild upper respiratory tract infection, 1 chronic myeloid leukemia. The one who developed leukemia is a male CD patient, with onset of Crohn disease at 13 years of age and previous therapies with steroids (8 courses), azathioprine (switched to 6-MP due to leucopenia) and who received infliximab (6 infusion over 9 months) concomitant to 6-MP due to fistulizing, chronically active disease; he used to be a drug-addicted man. Leukemia developed while he was on infliximab, in 2006. He underwent 5 surgeries with colectomy, repeated ileal resections leading to short bowel syndrome, and peristomal abscess drainage; he also had osteopenia.

Surgery for IBD was performed in 19/36 patients, prior to infliximab in 8 patients, after discontinuation of IFX in 5 patients (which did not eventually receive adalimumab), after discontinuation of infliximab and adalimumab in 6 patients.

Fourteen patients out of 19 undergoing surgery had CD: 7 underwent procedures before IFX therapy (perianal abscess drainage and fistulotomy with 1 concomitant ileal resection); 2 post-IFX discontinuation (1 had ileal resection and one had colectomy + multiple resections + perianal abscess drainage and fistulotomy); 5 after discontinuation of IFX and ADA (perianal abscess drainage and fistulotomy + colectomy).

Five patients out of 19 had ulcerative colitis: 4 underwent colectomy with IPAA (1 prior to IFX therapy, 2 after discontinuation of IFX, 1 after IFX and ADA), 1 perianal abscess drainage + fistulotomy after discontinuation of IFX.

The total number of surgical procedures was 40 (mean: 2.1 procedures per patient). Most surgeries were performed on patients who lost response to IFX, particularly after discontinuation of treatment; none of the responders needed surgery after IFX therapy (see table below):

Surgery (cases)	***Responders***	***Non-responders***	***Loss of response***
1	Pre-IFX	Post-IFX	Pre-IFX
2	Pre-IFX	Post-IFX	Post-IFX
3	Pre-IFX	Post-IFX	Post-IFX
4	Pre-IFX		Post-IFX
5			Post-IFX
6			Post-IFX
7			Post-IFX
8			Post-IFX and ADA
9			Post-IFX and ADA
10			Post-IFX and ADA
11			Post-IFX and ADA
12			Post-IFX and ADA

Adalimumab therapy

Twenty-one patients were treated with adalimumab, 15 males, 6 females. Nineteen out of 21 had Crohn disease, 1 ulcerative colitis, and 1 pouchitis.

Median age at diagnosis was 15 years (range 7-18 years, mean 14.4 years). Median disease duration was 3.6 years (mean 4.3 years, range 1-10.5 years). Five out of 21 patients (23.8%) received adalimumab before 18 years of age.

According to the Montreal Classification, Crohn disease was ileal (L1) in 1 patient, colonic (L2) in 2 patients, ileocolic (L3) in 13 patients; ilecocolic and upper GI (L3+L4) in 3 patients; there was no isolated upper GI (L4) involvement. Behavior type was inflammatory in 9 patients, stricturing in 3 patients, fistulizing in 6 patients, structuring and fistulizing in 1. Strictures developed prior to ADA therapy in 1 patient, prior to ADA but after IFX administration in one, after ADA treatment in 2 patients.

Ulcerative colitis was a pancolitis in 1 patient; the patient with pouchitis underwent a colectomy with ileal pouch-anal anastomosis for ulcerative pancolitis, and later developed a perianal fistula.

Extraintestinal manifestations were recorded in 5 patients (23.8%): 2 cutaneous (erythema nodosum), and 3 osteoarticular (2 arthralgias, 1 osteoporosis). One patient had as an associate disease a pituitary adenoma which was not influenced by adalimumab administration during follow-up *(Nobile S, et al. Adalimumab does not influence pituitary adenoma in a child with active refractory Crohn's disease. Dig Liv Dis 2009;41;3:S216).*

Eight patients (38.1%) had perianal disease: 7 had CD, 1 had pouchitis.

At the start of ADA therapy, 5 patients were receiving systemic steroids (2 of them could discontinue steroids while on adalimumab); 3 patients were assuming azathioprine/6MP (1 could discontinue AZA while on adalimumab).

Patients received adalimumab subcutaneously, every 2 weeks. The dose was calculated as mg/body surface area, by extrapolating from the recommended adult dosage: most patients received 80 mg/1.73 m^2 first dose, 40 mg/1.73 m^2 as maintenance dose.

Mean age at first dose was 18.6 years (median 19, range 13.5-25), mean duration of therapy 13 months (median 12, range 1-30).

Indications for biologic use were:

- chronically active disease resistant to other therapies: 19 patients, 17 of whom had received infliximab and eventually had loss of response or flare after discontinuation;
- steroid-dependency (2 patients).

The median duration of follow-up after the onset of adalimumab therapy was 19 months (mean 22.2 months, range 8-40 months).

We did not observe serious adverse events following adalimumab administration. In 9 patients, recorded adverse events were: skin reactions, pain to the site of injection, infections (one EBV infection, one dental abscess in one patient and a HPV infection in a girl).

Seven out of 21 patients (33.3%) reported complete remission of symptoms lasting for 22.7 months (mean; range 8-42) after ADA start; 12 patients (57.2%) reported improvement of symptoms for a mean duration of 18 months after ADA start (range 3-66); 2 patients (9.5%) did not experience any improvement.

Endoscopy was available for 11 patients: in 4 of them (36.4%) there was mucosal healing after a mean period of 22.2 months from ADA start (range 10-30); for 5 patients (45.4%) there was improvement but not endoscopic remission after a mean period of 13.8 months from ADA start (range 3-27); in 2 patients (18.2%) there was no endoscopic improvement.

Laboratory response was evaluated in 13/21 patients: C-reactive protein normalization (levels < 0.6 mg/dl) was evident in 7/13 patients (53.9%), and lasted for a mean duration of 17.9 months after ADA start (range 7-42); in 2/13 patients (15.4%) there was a reduction but not normalization of CRP levels; 4/13 patients (30.7%) did not show CRP reduction.

Eleven patients out of 21 underwent surgical procedures; 6/11 (54.5%) had surgery performed prior to ADA, while 5/11(45.5%) received surgery after ADA therapy: 4 had colectomy due to severe disease (1 was ulcerative colitis), 1 had fistulotomy. Surgery was performed after ADA therapy in 1/2 non-responders and in 5/10 patients who had loss of response; patients who achieved response did not receive post-ADA surgery.

Discussion

The efficacy and safety of infliximab in the treatment of adult patients with moderate to severe Crohn disease is well documented, and there are some studies that investigated the role of biologic therapy in pediatric IBD: most of this studies are open-label studies or case reports, while the largest cohorts come from multicentric studies.

A number of studies evaluated **infliximab** in pediatric IBD, mostly Crohn disease.

De Bie *(de Bie et al. The duration of effect of infliximab maintenance treatment in paediatric Crohn's disease is limited. Alim Pharmacol Ther 2011;33:243-250)* conducted an observational multicenter study on pediatric Crohn disease (152 patients who received a median number of 10.5 infusions over 16 months) with a median follow up of 25 months; 10% of patients had a prolonged response of at least 3 months after cessation of IFX therapy, 61% required repeated infusions, whereas 26% had loss of response and 3% had no response to treatment. Majority of prolonged responders was treated with IFX because of isolated fistulizing disease (9/15), and received a three-dose induction scheme only; of these, 7/15 remained in remission during a median follow-up of 18 months. The cumulative probability of losing response to IFX in patients who required repeated infusions was 13%, 40%, and 50% after 1, 3 and 5 years respectively. Half of patients required adjustments in treatment schedule: 43% of them required both a decrease in infusion interval and an increase in dosage. No predictors of IFX failure were found, and 45/152 patients (29%) experienced primary or secondary IFX failure; in most of them, maintenance treatment with immunomodulators was undertaken, while half of them eventually needed surgery after a median of 15.5 months from the first IFX infusion. Eleven percent of patients experienced a type 1 allergic reaction during infusion; 2% had recurrent reactions despite prophylaxis. Infections were seen in 16% of patients and were mild, except in two patients. Eight percent of patients had skin manifestations, one patient had basal cell carcinoma.

In the REACH study, 103 pediatric patients received a 3-dose IFX induction regimen and a maintenance therapy every 8 weeks or every 12 weeks interval; the two maintenance regimens were compared at 54 weeks from baseline, and the former was found to be more effective *(Hyams J, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863-873).* All patients had to be on concomitant immunosuppressors at least 8 weeks before screening. Mean disease duration was 1.6 years. Clinical response (using PCDAI) and safety were evaluated. 88% of patients responded to induction

at week 10, 59% being in remission; at week 54, 63% of randomized patients on the 8-week schedule were in clinical response and 56% were in remission (a statistically greater proportion versus the 12-week schedule). Significant decrease in corticosteroid use was observed throughout the study, and height z-scores improved. One-third of patients needed an increase of IFX dose and/or infusion frequency because of loss of response, and most of them regained response. Adverse events were infections (usually mild) in 74% of patients with the 8-week maintenance, and infusion reactions in 17% of patients. Another report from the REACH study evaluated 22 pediatric patients with concomitant luminal and perianal disease who were randomized to receive the same schedules of IFX as described above for a mean duration of 44 weeks and a mean follow-up of 52 weeks. Male gender and oral corticosteroids were found to be associated to perianal disease in the REACH cohort. Nine out of 22 (41%) patients achieved response at week 2 (4 partial and 5 complete; at weeks 10 and 54, 16/22 patients (73%) achieved response, with better results for those on the 8-week IFX schedule. Nine patients, however, developed perianal signs and symptoms and 2 of them responded at week 54. The concomitant use of corticosteroids, antibiotics, and immunosuppressants during IFX maintenance therapy did not add benefit to symptom relief.

According to Hyams et al, during the third year of IFX maintenance therapy, 33% of patients had sustained remission (defined as clinically inactive disease not requiring corticosteroids or surgery) *(Hyams J, et al. Long-term outcome of maintenance infliximab therapy in children with Crohn's disease. Inflamm Bowel Dis 2009;15:816-822).* De Ridder reported that sustained clinical response after cessation of IFX treatment or on IFX maintenance therapy was present in 70% of patients after a mean follow-up of 41 months *(de Ridder, et al. Infliximab dependency in pediatric Crohn's disease: long-term follow-up of an unselected cohort. Inflamm Bowel Dis 2008;14:353-358).*

A recent study by Malik conducted on 28 patients found a clinical response rate of 75% and remission rate of 36% at six months from IFX start, and a significant increase in linear growth among IFX responders *(Malik S, et al. Improvement in growth of children with Crohn disease following anti-TNF-α therapy can be independent of pubertal progress and glucocorticoid reduction. J Pediatr Gastroenterol Nutr 2011;52:31-37).*

A randomized, multicenter, open-label study by Ruemmele showed that IFX was well tolerated and safe as maintenance therapy in 40 children with CD, with a clear advantage when used on a scheduled 2-month basis compared to an "on demand" basis: 85% remission rate at week 10, 83% remission rate in the scheduled regimen group at 60 weeks follow-up *(Ruemmele FM, et al. Efficacy of Infliximab in Pediatric Crohn's Disease: A Randomized Multicenter Open-label Trial Comparing Scheduled to On Demand Maintenance Therapy. Inflamm Bowel Dis 2009;15:388 – 394).*

Our results are similar to those cited above, except for a higher rate of clinical response in our patients (despite lower endoscopic and laboratoristic response rates), when compared to the other studies. Endoscopic evaluation allows to demonstrate the "mucosal healing", often cited as the goal of treatment; however, endoscopy was not performed in the other studies, while is a strong point of our study.

	de Bie (2011)	Malik (2011)	REACH (2007)	Ruemmele (2009)	**Present study**
Patients	152	28	103	40	**36**
Responders	71% (clinical)	75% (clinical)	63% (clinical)	83% (clinical)	**91.7% (clinical), 66.7% (endoscopic), 61.4% (laboratoristic)**

Our patients were followed for a longer period of time compared to most studies (31.5 months versus 12 months). We did not observe serious adverse events, except for one case of chronic myeloid leukemia in a patient treated with concomitant immunosuppressants (azathioprine/6-MP). Fourteen patients receiving infliximab (38.9%) in our cohort suffered from perianal disease: most of them (10/14) initially responded to the drug but eventually had loss of response; 3/14 had complete resolution of symptoms; 1 had no improvement. Seven patients received surgical fistula correction prior to IFX, 2 after IFX, 3 of them after IFX and ADA, while 2 did not undergo surgery.

The total number of surgical procedures among patients treated with infliximab was 40. Most surgeries were performed on patients who lost response to IFX, particularly after discontinuation of treatment; none of the responders needed surgery after IFX therapy.

Because of either failure of medical management or development of adverse reactions to infliximab, **adalimumab** has emerged as the next choice in anti-TNF- α therapy, mainly for its recombinant human composition and for its ease of administration (subcutaneous injection).

Several trials of ADA therapy have been conducted in adults with CD: the CLASSIC-I trial demonstrated that adalimumab is superior to placebo in inducing remission of patients naïve to infliximab *(Hanauer SB, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology 2006;130:323-333);* the

CLASSIC-II and CHARME trials demonstrated that maintenance of remission of CD could be achieved on an every-week or every-other-week dosing schedule with adalimumab *(Sandborn WJ, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC-II trial. Gut 2007;56:1232-1239 , Colombel JF, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology 2007;132:52-65).* Other reports confirm the efficacy of adalimumab in adult Crohn disease and ulcerative colitis *(Sprakes M, et al. Adalimumab as second line anti-tumour necrosis factor alpha therapy for Crohn's disease: A single centre experience. JCC 2011;5:324-331 , Swoger JM, et al. Adalimumab for Crohn's Disease in Clinical Practice at Mayo Clinic: The First 118 Patients. Inflamm Bowel Dis 2010;16:1912–1921 , Barreiro-de Acosta M, et al. Adalimumab in ulcerative colitis: Two cases of mucosal healing and clinical response at two years. World J Gastroenterol 2009;15:3814-3816 , Sandborn WJ, et al. Adalimumab Induces and Maintains Clinical Remission in Patients With Moderate-to-Severe Ulcerative Colitis. Gastroenterology 2012;142:257–265).*

A few pediatric studies evaluating adalimumab efficacy in pediatric IBD have been published.

In a study by Rosenbach, 14 children were treated with ADA following IFX-failure for a median of 16.4 months and followed for 17.3 months; 7/14 had extraintestinal manifestations, 6/14 had perianal disease. Half of the patients showed a complete response, the other half a partial response; perianal disease showed healing in 2 patients and improvement in other two. Loss of response was recorded in two patients, and adjustment of ADA dose was needed in 8/14 children in order to maintain remission. At the start of ADA treatment, 13/14 were receiving immunosuppressants and had steroid-dependent or resistant disease; 8/14 could withdraw steroids. Overall, five patients were in complete remission with ADA monotherapy after a median follow-up of 14 months. Only one adverse event was recorded, that is abdominal abscess requiring surgery *(Rosenbach Y, et al. Adalimumab treatment in children with refractory Crohn's disease. Dig Dis Sci 2010;55:747-753).*

Viola evaluated 23 IBD patients treated with ADA, 9 naive and 14 intolerant or unresponsive to IFX. At weeks 24 and 48, remission rates were 50, and 65.2%, respectively, whereas response rates were 86, and 91%, respectively. Four patients at week 24 and 2 at week 48 received immunosuppressants; the mean daily corticosteroid dose, disease activity index, C-reactive protein level, and erythrocyte sedimentation rate decreased significantly throughout the study. *(Viola F, et al. Efficacy of adalimumab in moderate-to-severe pediatric Crohn's disease. Am J Gastroenterol 2009;104:2566-2571).*

Another report by Wyneski evaluated ADA in 14 patients with IFX-failure (due to allergic reactions or attenuated response), who received ADA for 6.5 months: 7/14 had a complete response, 2 had a partial response, and 5 had no response to treatment. Of the 5 patients with fistulizing

disease, 3 maintained fistula closure, 1 had temporary closure, and 1 required surgery. Only mild adverse events were recorded, specifically abdominal pain and nausea. Eight patients continued to receive azathioprine during adalimumab therapy *(Wyneski MJ, et al. Safety and efficacy of adalimumab in pediatric patients with Crohn disease. J Pediatr Gastroenterol Nutr 2008;47:19-25).*

We evaluated 21 patients with a median disease duration of 3.6 years. Indications for adalimumab treatment in the present cohort were: active disease resistant to other therapies (19/21) and steroid-dependency (2/21). Seventeen patients (81%) had previously received infliximab with loss of response or flare after infliximab discontinuation. Our results are compared to those of others in the table below:

	Rosenbach (2010)	Viola (2009)	Wyneski (2008)	**Present study**
Patients	14	23	14	**21**
Response rate	100% (clinical) at 17.3 months	91% at 12 months (clinical)	64% (clinical)	**91.5% (clinical) at 20 months, 81.8% (endoscopic), 69.3% (laboratoristic)**

Again, compared to other studies which did not show endoscopic results, we showed a high endoscopic response for patients receiving adalimumab.

We did not observe serious adverse events following adalimumab administration. In 9 patients, recorded adverse events were: skin reactions, pain to the site of injection, infections (one EBV infection, one dental abscess in one patient and a HPV infection in a girl).

Adalimumab therapy led to discontinuation of steroids in 2/5 patients (40%) receiving systemic steroids at the start of therapy. Three patients were assuming azathioprine/6MP, and one of these (33.3%) could discontinue azathioprine while on adalimumab.

Eight patients (38.1%) had perianal disease: 7 had CD, 1 had pouchitis. Of them, 3/8 achieved remission of symptoms, 4 had loss of response to ADA, 1 did not respond at all. Only one of these patients did not receive surgical fistula correction; 5/8 had surgical fistula correction prior to biologic therapy, 2 after adalimumab therapy.

Overall, 11/21 patients (52.4%) underwent surgical treatment of IBD; 6/11 underwent surgery before ADA treatment, while 5/11 patients after adalimumab therapy (4 colectomies for CD, 1 for UC). Surgical rates among our patients may be explained by their complex diseases, resistant to multiple therapies.

Limitations of the present study are the relatively small cohort of patients limiting the statistic significance of the results, and the retrospective nature of the observation.

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