



# UNIVERSITÀ DEGLI STUDI DI PALERMO

PhD Course in Molecular and Experimental Medicine  
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## The Effect of Probiotics in The Therapy of Ulcerative Colitis - a clinic study -

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## 1. Introduction

### 1.1 The Ulcerative Colitis

The Inflammatory Bowel Diseases (IBD) include two pathological entities of multifactor etiology not completely understood characterized by a complex immunological and inflammatory process in chronic form: Crohn's Disease and Ulcerative Colitis (UC).

In the context of IBD, a different form of ulcerative indefinite etiology, referred to as Indefinite Microscopic Colitis was also inserted.<sup>1</sup>

In particular, in the preparation of this thesis, attention has been made to the UC whose chronic inflammatory process seems to depend on genetic predisposition and epigenetic environmental factors are not yet completely known.



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<sup>1</sup> Strober W, Fuss I, Manon P, De Foundmental Basis of IBD. J Clin Invest 2007; 117:

## 1.2 Epidemiology

The highest rates of incidence and prevalence of UC are recorded in North America and Northern Europe, with an impact ranging from 9 to 20 new cases a year per 100,000 individuals and the prevalence rates range from 156 to 291 cases per 100,000 individuals.<sup>2</sup>

The recorded data indicates a higher impact in industrialized countries confirming the extremely important role exercised by environmental factors in the genesis of chronic inflammatory process, typical of the disease.

The UC reflects a characteristic bimodal pattern of incidence, with a peak incidence occurring between the ages of 15 and 30 and a second less significant peak of incidence between the ages of 50 and 70 with effects slightly higher in males than females.<sup>3</sup>

The onset cases at an early age account for approximately 30% and similar to that observed in adulthood, recent studies show a clear increase of the disease in children.<sup>4</sup>

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<sup>2</sup> Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative Colitis. *Lancet* 2012; 380: 1606-1619

<sup>3</sup> Loftus EU JR, Sandborn WJ, Epidemiology of IBD. *Gastroenterol Clin Nord AM* 2002; 31: 1-20

<sup>4</sup> Pappa HM, Semrin G, Walzer TR, Grand RJ. Pediatric Inflammatory Bowel Disease. *Curr Opin Gastroenterol* 2004; 20: 33-40

### 1.3 Etiopathogenetic Mechanisms

The UC, a chronic inflammatory bowel disease in which etiopathogenesis is not yet well defined, is certainly multifactorial. The UC consider the most accredited etiopathogenetic hypothesis, as the result of the influence of several environmental epigenetic factors on a predisposed genotype, capable synergetically, to interact with the host immune system.

In this way, an inappropriate and persistent activation of the immunitary response at the level of the intestinal mucosa would trigger, facilitated in turn, by a pre-existing impairment of barrier functions. The latter would be due to a reversal of the ultra structural junction of the mucosal system.

The result of these complex mechanisms would give rise to the characteristic inflammatory phenotype of the UC.<sup>5</sup>

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<sup>5</sup> Hanauer SB. Inflammatory Bowel Disease: Epidemiology, Pathogenesis And Therapeutic Opportunities. Inflamm Bowel Dis 2006: 12S1

### 1.3.1 Genetic Factors

Numerous epidemiological studies largely confirmed that the UC and in general all the IBD, are genetically complex diseases characterized by high genetic heterogeneity, able to interact with specific environmental factors affecting the phenotypic characteristic appearance of the disease.

Hereditary studies show that about 5.7-15.5% of individuals with IBD have a first-degree relative affected by UC or CD.<sup>6</sup>

The familial aggregation observed in IBD, confirms the hypothesis of the presence of a genetic susceptibility, on which environmental factors contribute to the onset of the disease.

Numerous studies on monozygotic and dizygotic twins, outline the different influence of genetic profile and environmental factors on etiopathogenesis of UC and CD.

In particular, studies show concordance rates for UC significantly higher for monozygotic twins (6-17%) compared to dizygotic twins (0-5%), confirming the role played by genetic factors.<sup>7</sup>

The relationship between patients with UC and unaffected members within the same family, suggests a hereditary pattern that differs significantly from the classical Mendelian model with complete penetration, confirming the complex heterogeneity of the disease.

Recent Linkage studies have made it possible to acquire more precise information on IBD genetic heterogeneity, identifying locus chromosomal susceptibility and candidate genes for both the CD and UC.

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<sup>6</sup> Farmer RG, Michener WM, Mortimer EA. Studies of Family History Among Patients with IBD. *Clin Gastroenterol* 1980; 9: 271-277

<sup>7</sup> Satsangi J, Morecroft J, Shah NB. Genetics of Inflammatory Bowel Disease: Scientific and Clinical Implications. *Best Pract Res Clin Gastroenterol* 2003; 17: 3-18



In particular, genes responsible for susceptibility to IBD in general exist, disease-specific genes that can influence the onset of CD or UC and phenotype-specific genes that influence the location, the natural history of the disease and the different individual capacity to respond to therapeutic treatment.

Linkage studies of the whole genome has allowed the identification of several locus susceptibility for IBD.<sup>8</sup> (Table 1)

Locus	Chromosome	Disease
IBD 1	16q12	MC
IBD 2	12p13-q24	CU
IBD 3	6p	CU, MC
IBD 4	14q11-12	MC
IBD 5	5q31-33	MC
IBD 6	19p13	MC, CU
IBD 7	1p36	MC, CU

Table 1: Genetic Linkage Studys and candidate genes. IBD Year Book 2005

Additional studies have identified other chromosomal areas with greater evidence of linkage to UC.<sup>9</sup> (Table 2)

<sup>8</sup> Brant SR, Shugart YY. Inflammatory Bowel Disease Gene Hunting by Linkage Analysis. Rationale Methodology and Present status of the Field. *Inflamm Bowel Dis* 2004i; 10: 300-311

<sup>9</sup> Van Heel Da, Fisher SA, Kirby A, Daly MJ Riouxjd, Lewis CM. Inflammatory Bowel Disease Susceptility Loci Defined by Genome Scan Meta-Analysis of 1592 Affected Relative Pairs. *Hum Mol Genet* 2004; 13: 763-770

Locus	IBD	CD	UC
2q24-q34	0.004	0.04	0.01
3q27-qter	0.005	0.02	
4q23-q28			0.05
4q28-q32			0.04
5q13-q15	0.02		
6pter-p23	0.005	0.01	
6p23-p21.1 (IBD3)	0.0001	0.004	0.05
7q11.1-q21	0.02		
12p12-q13 (IBD 2)	0.05	0.04	0.02
16p13.1-q12.2 (IBD 1)	0.008	0.003	
16q12.2-q22		0.01	
17q21-q24		0.01	
19pter-p13.2		0.007	
19p13.2-q13.2 (IBD 6)		0.01	
19q13.2-qter			0.03

Table 2: Chromosomal Locus and Genetic Linkage. IBD Year Book 2005

### 1.3.2 Environmental Factors

Although the existence of a genotype susceptibility and specific "candidate genes" in IBD was confirmed, several studies emphasize the importance of environmental factors that can provoke or act as protector in the UC.

Breastfeeding for example, especially if more than 3 months, certainly acts as a protective factor against the UC.<sup>10</sup>

Smoking also appears to play a protective role for RCU. In fact, studies show that the course of the disease seems to be slower in smokers than non-smokers.<sup>11</sup>

Previous episodes of bacterial gastroenteritis supported by *Campylobacter*, *Salmonella* and *Shigella* can determine a significant modification of the intestinal flora by acting as triggers on the onset of the chronic inflammatory process of IBD.<sup>12</sup>

Numerous studies have previously demonstrated the protective role for UC of appendectomy especially if the person is subjected to surgery within the first 20 years of age.<sup>13</sup>

The scientific literature data show a 69% reduction in the risk of developing UC after appendectomy.<sup>14</sup>

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<sup>10</sup> Klement E, Cohen RV, Boxman J, Joseph A, Reif S. Breastfeeding and Risk of Inflammatory Bowel Disease: A Systematic Review with Meta-Analysis. *AM J Clin Nutr* 2004; 80: 1342-1352

<sup>11</sup> Ordas I, Eckmann L, Talamini N, Baumgart DC, Sandborn J. Ulcerative Colitis. *Lancet* 2012; 380: 1606-1619

<sup>12</sup> Garcia Rodriguez LA, Rui Gomez A, Panes J. Acute Gastroenteritis is Followed by An Increased Risk of Inflammatory Bowel Disease. *Gastroenterology* 2006; 130: 1588-1594

<sup>13</sup> Duggan AE, Usmani I, Neal KR, Logan RF. Appendectomy, Childhood Hygiene, H. Pylori Status and Risk of Inflammatory Bowel Disease: A Case-Control Study. *Gut* 1998; 43: 494-498

<sup>14</sup> Kout Roubakis IE, Vlachonikolis IG. Appendectomy and the Development of UC: Results of a Meta-analysis of Published Case Control Studies. *AM J Gastroenterol* 2000; 95: 171-176

Subsequent studies have confirmed this protective role of appendectomy in the onset of UC proving even a phenotype disease significantly less aggressive than in UC patients not undergoing appendectomy.<sup>15</sup>

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<sup>15</sup> Randford- Smith GL, Edwards JE, Purdie DM, Pandeya N, Watson M, Martin NG. Protective Role of Appendectomy on Onset and Severity of Ulcerative Colitis and Crohn's Disease. Gut 2002; 51: 808-813

## 1.4 Pathophysiological Mechanisms

The altered intestinal permeability, which originated from the destruction of the junctional mucosa (TJ) and the mucus film that covers the epithelium, in the presence of significant alterations of the microbiota, stimulate a disordered local immune response, by producing pro-inflammatory factors and recruiting specific cellular elements.

This should be a summary of the pathophysiological framework that underlies the characteristic chronic inflammatory process of IBD. (Fig.1)

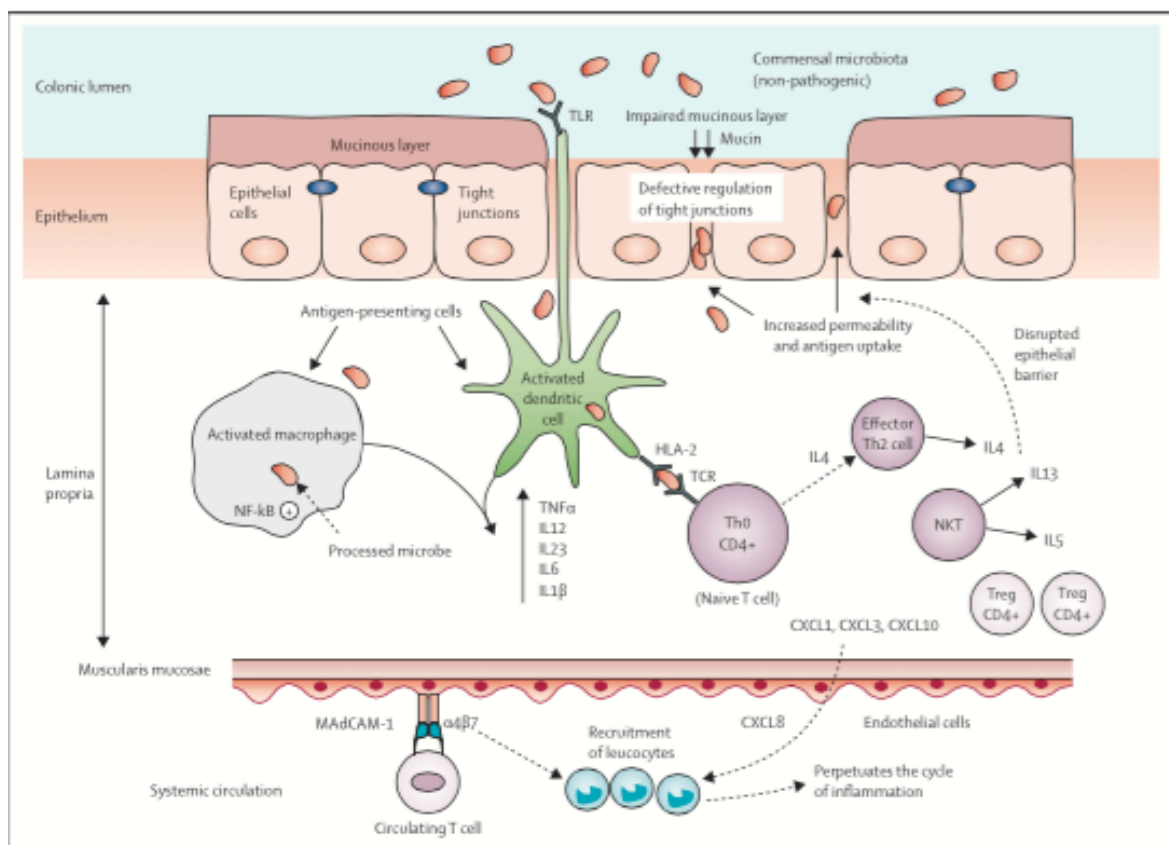


Fig. 1 Pathophysiology of Ulcerative Colitis. Ingrid Ordás, Lars Eckmann, Mark Talamini, Daniel C Baumgart, William J Sandborn. *Ulcerative colitis. Lancet* 2012; 380: 1606–19

### 1.4.1 Intestinal Microbiota

Several studies published in recent years have confirmed the role of the intestinal microbiota as an important epigenetic factor that can affect the appearance of specific modifications of the intestinal micro-environmental characteristics (intestinal permeability, altered cytokine network) resulting in inappropriate bacterial translocation and persistent activation of the local immune response widely recognized in patients suffering from IBD.

The presence of persistent intestinal dysmicrobism characterized by qualitative and quantitative alterations of the normal resident bacterial flora, and the presence of a microbial pathogen load in a position to contribute significantly to the onset of the typical inflammation process of IBD is observed in patients with CD and UC.<sup>16</sup>

The hypothesis of an intestinal dysmicrobism typical of patients with UC is supported by studies showing the up-regulation of the expression of beta-defensins (Beta-Defensin 2).<sup>17</sup>

It has been demonstrated that, in patients with IBD, the intestinal microbiota loses the ability to down-regulate inflammatory genes and to inhibit the activation of NF- $\kappa$ B by triggering an inflammatory response loading the cells of the intestinal mucosa so as to encourage a chronic alteration of the immune response.<sup>18</sup>

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<sup>16</sup> Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, Leleiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the Intestinal Microbiome in Inflammatory Bowel Disease and Treatment. *Genome Biology* 2012; 13: R79

<sup>17</sup> Rahman A, Fahl Gren A, Sundstedt C, Hammarstrom S, Danielson A, Hammarstrom MI. Chronic Colitis Induces Expression of Beta-Defensins in Murine Intestinal Epithelial Cells. *Clin Exp Immunol* 2011; 163: 123-130

<sup>18</sup> Colletti T. IBD-Recognition, Diagnosis and Therapeutics. *JAAPA* 2004; 7: 16-24

The intestinal microbiota is a complex ecosystem characterized by the coexistence of diverse ecological niches, which are home to a microbial population, consisting of numerous bacterial species and strains.

Recent advances in molecular biology techniques applied to genomic sequencing and studies of Metagenomics, have allowed us to make a more precise and detailed classification of microbiota.

In this way, it was possible to greatly expand the knowledge already acquired, both in terms of its genotypic characterization and of its genotypic characteristics, and potential metabolic capacity to exercise it.

The results of two major research projects, the Human Microbiome Project (HMP), performed in the USA, and the great Metagenomics of Human Intestine (METAHIT), taking place in Europe, have expanded the knowledge about the composition of the bacterial flora present along our entire gastrointestinal tract, allowing to typify over 1100 bacterial species and over 15000 different bacterial strains, able to constitute a biomass of about 1 Kg.<sup>19</sup>

In the light of present knowledge, approximately 90% of the intestinal microbial population is represented by 2 dominant phylum formed by Bacteroidetes (Bacteroides and Prevotella), while the remaining 10% is variously divided into 5 sub-dominant phylum: Actinobacteria, Proteobacteria, Fusobacteria, Cyanobacteria and Verrucomicrobia.<sup>20</sup>

Subsequent studies have identified a specific "enterotype" characteristic of our species, likely to meet a qualitative and quantitative modifications in the natural course of life to constitute a different enterotype, both phylogenetic

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<sup>19</sup> Qin J, Li R, Raes J, Arumugam M. A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing. *Nature* 2010; 4, 464: 59-65

<sup>20</sup> Kuczynski J, Costello EK, Nemergui DR, Zaneveld J, Lauber CL, Knigcts D, Coren O, Fierer N, Kelley ST, Ley RE, Gordon JI, Knight R. Direct Sequencing of the Human Microbioma Readily Reveals Community Differences. *Genome Biology* 2010; 11: 210

and functional terms, capable of preparing the subject towards specific pathological conditions.<sup>21</sup>

Studies in mouse models have shown the possibility of enterotype to evolve towards a predominant variant: "enterotype 2" (Bacteroidetes).

This eventuality is positively correlated with a high concentration of fecal Calprotectin, known index of the intestinal mucosa, found in high concentrations in patients with UC. Previous studies have allowed the identification of a modification of the intestinal microbiota, both in patients with CD and UC.<sup>22</sup>

In particular, some studies show a clear reduction of Lactobacilli and Bifidobacteria, in histological specimens and cultivation of patients with UC.<sup>23</sup> The reduction of the normal intestinal flora, is accompanied by the bacterial pathogen overgrowth responsible for carrying out the pro-inflammatory action in consideration of the intestinal mucosa with consequent onset of UC.<sup>24</sup>

A demonstration of bacterial pathogen overgrowth of pro-inflammatory action found in IBD stems from the discovery in the urine of patients of specific metabolites (Hippurate hydrolases), produced by bacterial fermentation processes.<sup>25</sup>

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<sup>21</sup> Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende RD, et al. Enterotypes of the Human Gut Microbiome. *Nature* 2011; 473: 174-180

<sup>22</sup> Hildebrand F, Brinkman B, Yunta RG, Cauwe B, Vandenabeele P, Liston A, Raes J. Inflammation-Associated Enterotypes, Host Genotype Cage and Inter-Individual Effects Drive Gut Microbiota Variation in Common Laboratory Mice. *Genome Biology* 2013; 14: R4

<sup>23</sup> Ouwshand AC, Salminen S, Roberts PJ. Disease-Dependent Adhesion of Lactic Acid Bacteria to the Human Intestinal Mucosa. *Clin Diagn Lab Immunol* 2003; 10 (4): 643-646

<sup>24</sup> Gionchetti P, Rizzello F, Lammers KM. Antibiotics and Probiotics in Treatment of IBD. *World J Gastroenterol* 2006; 12: 3306-3313

<sup>25</sup> Williams HR, Cox IJ, Walker DG. Characterization of IBD with Urinary Metabolic Profiling. *Am J Gastroenterol* 2009; 104 (6): 1435-1444



In terms of bacterial phylum, recent metagenomic studies carried out on individuals with IBD, detect a significant alteration of the two dominant phylum: Bacteroidetes and Firmicutes.<sup>26</sup>

It has also been demonstrated recently that a significant reduction of bacterial strains belonging to the genus *Roseburia* and *Phascolarctobacterium*, strains responsible to the production of short chain fatty acids (SCFA) in a particular way of butyrate and propionate, important in terms of trophism and protection of intestinal mucosa and to stimulate the production of regulatory T cells to anti-inflammatory action.<sup>27</sup>

It is also found in patients suffering from UC, a decreased concentration of bacterial species belonging to the genus *Leuconostocaceae* producing acetate and lactate.<sup>28</sup>

A significant reduction of concentration also affects the *Odoribacter* genus belonging to the family of *Porphyromonadaceae*.

It can be observed especially in patients with Pancolitis, a more severe clinical form of UC.

The reduced presence of these bacterial strains lays, not only in favor of an intestinal dysbiosis, but also in favor of metabolic modifications, of a reduced production of acetate in particular, propionate and butyrate, able to influence the inflammatory process of the intestinal mucosa.<sup>29</sup>

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<sup>26</sup> Frank DN et al. Molecular-Phylogenetic Characterization of Microbial Community in Balances in Human Inflammatory Bowel Diseases. *Proc Natl Acad Sci USA* 2007; 104: 13780-13785

<sup>27</sup> Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwattara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y. Induction of Colonic Regulatory T Cells by Indigenous *Clostridium* Species. *Science* 2011; 331: 337-341

<sup>28</sup> Cogan JG, Jordan KN. Metabolism of *Leuconostoc* Bacteria. *J Dairy Sci* 1994; 77: 2704-2717

<sup>29</sup> Goker M, Gronow S, Zeytun A, Nolan M, Lucas S, Lapidus A, Hammon N, Deshpande S, Cheng JF, Pitluck S. Complete Genome Sequence of *Odoribacter Splanchnicus* Type Strain (1651/6). *Stand Genomic Sci* 2011; 4: 200-209

The SCFA and, in particular, the butyrate, have shown to possess anti-inflammatory action but also to inhibit the processes of bacterial translocation.<sup>30</sup>

Subsequent studies have highlighted the close relationship between butyrate and barrier function of the intestinal mucosa.

Research shows in particular how the butyrate is able to regulate the expression of Claudin-2.<sup>31</sup>

The metabolic changes resulting from alterations typical of the intestinal microbiota of patients with IBD, does not only involve the short-chain fatty acids (SCFA), but also the metabolism of Glutathione synthesized by Proteobacteria, Streptococcus and Enterococcus, responsible to actively combat oxidative stress load of intestinal epithelial cells found in IBD.<sup>32</sup>

In fact, high concentrations of highly reactive species of oxygen and nitrogen in patients with CD and UC are found.<sup>33</sup>

To further confirm the role played by the intestinal flora in the UC, it is noted in the in vitro studies that the bacterial residents, due to high adhesion and invasiveness, are capable of inducing the production of toxins and pro-inflammatory mediators (IL -1, IL-6, IL-8) responsible for the transmigration

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<sup>30</sup> Lewis K, Lutgendorff F, Phan U, Soderholm JD, Sherman PM, McKay DM. Enhanced Translocation of Bacteria Across Metabolically Stressed Epithelia is Reduced by Butyrate . *Inflamm Bowel Dis* 2010; 16: 1138-1148

<sup>31</sup> Ploger S, Bergann T, Fromm A, Bucker R, Fromm M, Schulzke JD. Effect of Sodium Butyrate on Barrier Function of HT-29/B6-TET-ON-GR-MR Cells in Conjunction with Down Regulation of Claudin-2. *Genes Nutr* 2010; 5 (S1): S28

<sup>32</sup> Sherrill C, Fahey RC. Import and Metabolism of Glutathione by Streptococcus Mutans. *J Bacteriol* 1998; 180: 1454-1459

<sup>33</sup> Keshavarzian A, Banan A, Farhadi A, Komanduri S, Mutlu E, Zhang Y, Fields JZ. Increases in Free Radicals and Cytoskeletal Protein Oxidation and Nitration in the Colon of Patients with Inflammatory Bowel Disease. *Gut* 2003; 720-728

of neutrophils across the intestinal epithelium with the appearance of the typical crypt abscesses of the UC.<sup>34</sup>

Subsequent studies confirm that the HLA-B27 transgenic mice and mice deficient in IL-2 and IL-10 develop chronic colitis.<sup>35</sup>

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<sup>34</sup> Meijssen MAC et al. Altered Cytokine and CD14 Expression by Intestinal Epithelial Cells of IL-2 Deficient Mice. *Gastroenterology* 1996; 110: A966

<sup>35</sup> Rath HC. Role of Commensal Bacteria in Chronic Experimental Colitis: Lessons from the HLA-B27 Transgenic Rat. *Pathobiology* 2002; 70: 131-138

## 1.4.2 Intestinal Mucus

The gastrointestinal epithelium is continuously exposed to the insult exercised by a number of chemical, physical and biological agents.

The intestinal mucus represents an important defensive barrier of the epithelium against the potential harmful effect exerted by these agents.

It appears to be variously distributed along the various sections of our gastrointestinal system, arranged in a double stratification: an inner layer perfectly adherent to the epithelial surface and almost free of bacteria, and an outer layer in direct contact with the intestinal lumen.<sup>36</sup>

Its adequate physico-chemical composition and its perfect layering throughout the epithelium, are the essential elements for effective barrier action against damaging agents.<sup>37</sup>

The mucus produced by goblet cells, which are present in the intestinal epithelium, is richly peppered with mucins or glycosylated proteins that impart trophic lubricity and are protective towards epithelium.<sup>38</sup>

It is detected in patients suffering from UC a reduced representation of goblet cells, of the quantities of mucus and the amount of mucin that it forms. In fact, it has been demonstrated in IBD and in particular in the UC, the reduced expression of specific genes responsible for the adequate more or less adequate expression of goblet cells.<sup>39</sup>

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<sup>36</sup> O'Hara AM, Shanahan F. The Gut Flora as a Forgotten Organ. *EMBO* 2006; 7: 688–693

<sup>37</sup> Matsuo K at al. Histochemistry of the Surface Mucous Gel Layer of the Human Colon. *Gut* 1997; 40: 782-789

<sup>38</sup> Johansson ME at al. The Two Mucus Layer of Colon are Organized by the Muc2 Mucin, Whereas the Outer layer is a Legislator of Host-Microbial Interactions. *Proc Natl Acad Sci USA* 2011; 108 (S1): 4659-4665

<sup>39</sup> Gersemann M, Becker S, Kubler I. Differences in Goblet Cell Differentiation Between Crohn's Disease and UC. *Differentiation* 2009; 77 (1): 84-94

It is witnessed in the mucin deficient mice (MUC-2) or with altered glycosylation patterns, a major pathogenic bacterial proliferation and tendency to spontaneous colitis and colitis induced.

This study confirms the importance of an adequate production of mucus and a rich amount of mucin in countering the onset of mucosal intestinal inflammation.<sup>40</sup>

GF animal studies demonstrate a quantitative reduction of the goblet cells and the thickness of the mucus layer in colonic level, confirming the important role exercised by the intestinal bacterial flora in the maintenance of the physical and chemical mucus characteristics, of its quantity produced and its functional potentials.<sup>41</sup>

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<sup>40</sup> Fu J at al. Loss of Intestinal Core1-Derived O-Glicans Causes Spontaneous Colitis in Mice. *J Clin Invest* 2011; 121: 1657-1666

<sup>41</sup> Sharma R at al. Rat Intestinal Mucosal Responses to A Microbial Flora and Different Diets. *Gut* 1995; 36: 209-214

### 1.4.3 Junctional Mucosal System

Under physiological conditions, the intestinal epithelium plays an efficient barrier function allowing the transportation of selective paracellular and transcellular of specific ions and molecules, thanks to the intervention of highly specialized structures, which together constitute the defense system of the intestinal mucosa.

This system is responsible in maintaining an appropriate pattern of intestinal permeability.<sup>42</sup>

The structural alteration of this complex defense system involves the onset of a state of altered permeability, contributing to the onset of intestinal and extra intestinal pathological conditions.

Despite the immune dysregulation, inflammation and mucosal barrier function abnormalities are common factors found in patients with IBD, it is not yet clear which of these three mechanisms actually precedes the onset of the disease. Studies show that the only structural alteration of the defense system of the mucosa with consequent increased of intestinal permeability is not able to trigger the onset of IBD.<sup>43</sup>

On the contrary, the appearance of altered intestinal permeability is accompanied by immune dysregulation.

The defense system of the intestinal mucosa, from the structural point of view, consists of structures of a protein nature, adequately distributed along the side of the apical, basal and latero-lateral of epithelial cells (tight junction,

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<sup>42</sup> Turner JR. Molecular Basis of Epithelial Barrier Regulation From Basic Mechanisms to Clinical Application. *AM J Pathol* 2006; 169: 1901-1909

<sup>43</sup> Edelblum KL, Turner JR. The Tight Junction in IBD: Communication Breakdown. *Curr Opin Pharmacol* 2009; 9(6): 715-720

adherens Junction, maculae adherens, gap Junction, hemidesmosomes).  
 (Fig.2)

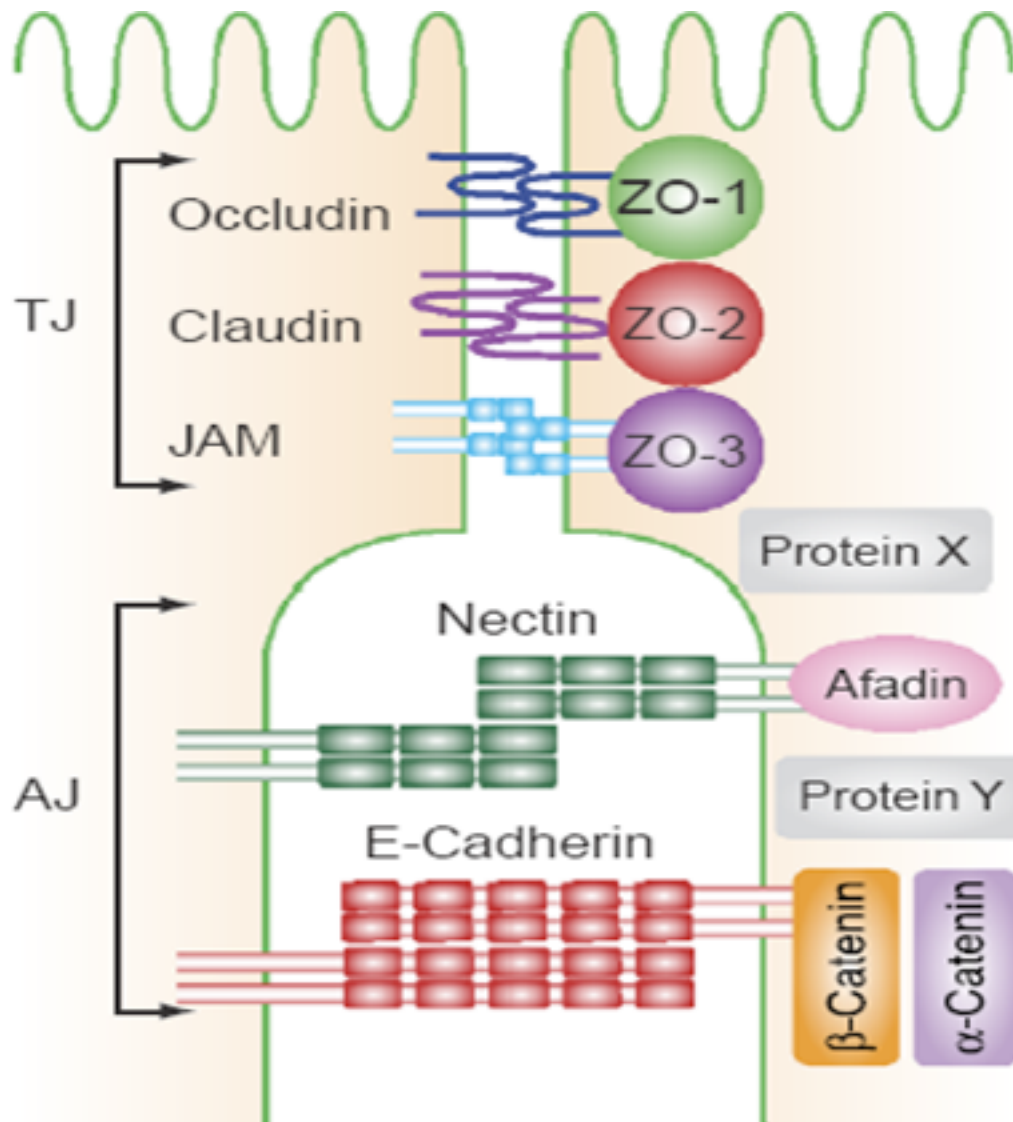


Fig. 2: Schematics showing composition and organization of adherens junctions (AJs) and tight junctions (TJs). JAM: Junction Adhesion Molecule. J. Miyoshi, Y. Takai, *Advanced Drug Delivery Reviews*, 2005)  
 From the biomolecular point of view, it deals with the complex structures made up of specific components of protein-based nature in a trans membrane confinement, among which Junctional Adhesion Molecules (JAM), Tricellulina, Occludin and Claudina can be distinguished.

Through their intracellular component, they are capable of interacting closely with specific membrane proteins among which Zonuline and Cinguline can be distinguished.<sup>44</sup>

The transcriptional processes of these complex protein molecules, constitutive of the mucosal defense system and responsible for its barrier function may be due to specific genomic sequences.

It has been demonstrated in patients with IBD a reduced expression of these proteins due to alterations of some genomic sequences.<sup>45</sup>

There is a reduced expression of Occludin in the gut epithelium in IBD.<sup>46</sup>

Further studies have also demonstrated the presence of altered transcriptional regulation and consequent altered expression of Claudine.<sup>47</sup>

The alterations of the defense system of the intestinal mucosa, responsible for determining the appearance of increased permeability in IBD, are directly attributable to dysregulated expression of specific pro-inflammatory cytokines: TNF-ALPHA, GAMMA-INF, IL-1beta and IL-13.<sup>48</sup>

In this regard, recent studies have made it possible to distinguish the various forms of IBD, the cytokine profile characteristic and specific alterations of the proteins of the defense system of the intestinal mucosa. (Tab. 3)

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<sup>44</sup> Mitic LL, Anderson JM. Molecular Architecture of TJ. *Ann Rev Physiol* 1998; 60: 121-142

<sup>45</sup> Mees ST, Mennigen R, Spieker T. Expression of Tight and Adherens Junction Proteins in UC Associated Colorectal-Carcinoma Up-Regulation of Claudin-1, 3, 4 and Beta-Catenin. *Int J Colorectal Dis* 2009; 24(4): 361-368

<sup>46</sup> Heller F, Florian P, Bojarski C, Richter J, Christ M, Hillenbrand B, Makertz J, Gitter AH, Burgel N, From M. Interleukin-13 is the Key Effector TH2 Citokine in UC that Affects Epitelial TJ, Apoptosis and Cell Restitution. *Gastroenterology* 2005; 129: 550-564

<sup>47</sup> Prasad S, Mingrino R, Kaukinen K, Hayes KL, Pouel RM, Mac Donald, Collins JE. Inflammatory Processes have Differential Effects on Claudins 2, 3 and 4 in Colonic Epitelial Cells. *Lab Invest* 2005; 85: 1139-1162

<sup>48</sup> Hering NA, Fromm M, Schulzke JD. Determinats of Colonic Barrier Function in IBD And Potential Therapeutics. *J Physiol* 2012; 5: 1035-1044



Inflammatory bowel diseases	Cytokine profile	Epithelial resistance ( $\Omega \text{ cm}^2$ )	Tight junction proteins	Other pathomechanisms	References
Crohn's disease (sigmoid colon)	TNF $\alpha$ , IFN $\gamma$ (Th1)	23 $\pm$ 3 (59% of control) (active, mild to moderate inflamed)	Claudin-2 $\uparrow$ Occludin $\downarrow$ Claudin-3 $\downarrow$ Claudin-5 $\downarrow$ and redistributed Claudin-8 $\downarrow$ and redistributed	Epithelial apoptosis $\uparrow$	Zeissig <i>et al.</i> 2007
Ulcerative colitis (sigmoid colon)	IL-13, TNF $\alpha$ (Th2)	20 $\pm$ 3 (21% of control) (active, mild to moderate inflamed)	Claudin-2 $\uparrow$ Occludin $\downarrow$ Claudin-1 $\downarrow$ Claudin-4 $\downarrow$ Tricellulin $\downarrow$	Epithelial apoptosis $\uparrow$ Ulcerations Microerosions	Heller <i>et al.</i> 2005; Schmitz <i>et al.</i> 1999a; Krug <i>et al.</i> 2010
Collagenous colitis (sigmoid colon) (microscopic colitis)	TNF $\alpha$ , IFN $\gamma$ (Th1)	29 $\pm$ 2 (23% of control) (macroscopically normal)	Claudin-2 $\uparrow$ Claudin-4 $\downarrow$ Occludin $\downarrow$	Malabsorption	Burgel <i>et al.</i> 2002; Tagkalidis <i>et al.</i> 2007

Table 3: Aspects of barrier dysfunction in IBD. Nina A. Hering, Michael Fromm, Jorg-Dieter Schulzke. J Physiol 2012; 590.5: pp 1035–1044

The alterations of barrier function may also be due to the intestinal dismicrobism. Recent studies have in fact demonstrated in mouse models deficient in IL-2 or IL-10 suffering from chronic inflammatory bowel condition that the transition to a GF, does not lead to the appearance of clinical symptoms typical of chronic inflammation.<sup>49</sup>

Subsequent studies demonstrate the involvement of Junctional Adhesion Molecule (JAM-A) in regulating the state of permeability of the intestinal mucosa and inflammation. (Fig. 2)

JAM-A is a protein associated with TJ, which is extremely important in terms of adjustment of the barrier function and, where the reduced expression is

<sup>49</sup> Elson CO, Cong Y, Mc Cracken VJ, Dimmitt RA, Lorenz RG, Weaver T. Experimental Models of IBD Reveal Innate, Adaptive and Regulatory Mechanisms of Host Dialogue with the Microbiota. Immunol Rev 2005; 206: 260-276

accompanied by high intestinal permeability, recruitment and activation of leukocytes with consequent increase in the mucosal inflammatory process.<sup>50</sup>

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<sup>50</sup> Laukoetter GM, Nava P, Lee YW, Severson EA, Capaldo CT, Babbin BA, Williams IR, Koval M, Parkos C. JAM-A Regulates Permeability and Inflammation in the Intestine in – Vivo. JEM 2007; 204 (13): 3067-3076

#### 1.4.4 Cytokine Network

The impressive and chronic inflammatory process seen in mucosal UC and in general in IBD, is the result, as a co-factor, also of alterations of the cytokine network.<sup>51</sup>

In particular, it has been demonstrated the presence of a richly peppered epithelium highly pro-inflammatory and damaging cytokines, in which the concentration depends on the extent of the inflammatory process characteristic of the UC.<sup>52</sup>

A widely-related cytokine, in this sense, is definitely the Tumor Necrosis Factor Alpha (TNF-A).

In fact, in patients with UC, it is detected a high concentration of TNF-Alpha in serum, faeces and at the level of the intestinal mucosa.<sup>53</sup>

Studies show that the magnitude, extent and progression of mucosal inflammation, is significantly related to the concentration of TNF-Alpha.

The impressive role played by TNF-A in the pathogenesis of chronic inflammatory process of IBD, has stimulated the execution of countless studies, wherein data have led to a breakthrough in the treatment of these diseases with the advent of therapies that exploit the action of specific antibodies monoclonal anti-TNF, with very encouraging results.<sup>54</sup>

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<sup>51</sup> Schulzke JD, Plogers S, Amasheh M. Epithelia TJ in Intestinal Inflammation. *Ann NY Acad Sci* 2009; 1165: 294-300

<sup>52</sup> Jalocha L, Wojtun S, Dyrła P. TNF-Alpha Polymorphism and Course of UC. *Pol Merkur Lekarski* 2009; 26(155): 444-445

<sup>53</sup> Komatsu M, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N. TNF-Alpha in Serum of Patients with IBD as Measured by a Highly Sensitive Immuno-PCR. *Clin Chem* 2001; 47: 1297-1301

<sup>54</sup> Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-TNF Monoclonal Antibodies in IBD: Pharmacokinetics-Based Dosing Paradigms. *Clin Pharmacology and Therapeutics* 2012; 1-11

Several studies show also an involvement in the inflammatory process of IL-32. It is a cytokine synthesized by monocytes, natural killer cells, T lymphocytes and epithelial cells.<sup>55</sup>

In particular, IL-32 is able to stimulate the synthesis of pro-inflammatory cytokines and chemokines thanks to the intervention of NF-KB and MAPK thus, stimulating the onset of IBD.<sup>56</sup>

The epithelial cells of the intestinal mucosa of these patients show a high concentration of a specific variant of IL-32: IL-32 Alpha.<sup>57</sup>

Other cytokines seem to play an important role in the pathophysiology of IBD and, in this sense, studies show an increased concentration of IL-17.<sup>58</sup>

The same pro-inflammatory role seems to be played also by IL-31, which is also increased in the CD and in the UC.<sup>59</sup>

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<sup>55</sup> Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA. IL-32: a Cytokine and Inducer of TNF-Alpha. *Immunity* 2005; 22: 131-142

<sup>56</sup> Shioya M, Nishida A, Yagi A, Ogawa A, Tsujikawa T, Kim-Mitsuyama S, Takayanagi A, Shimizu N, Fujiyama Y, Andoh A. Epithelial Overexpression of IL-32 Alpha in IBD. *Clin Exp Immunol* 2007; 149: 480-486

<sup>57</sup> Andoh A, Yagi Y, Shioya M, Nishida A, Tsujikawa T, Fujiyama Y. Mucosal Cytokine Network in IBD. *World J Gastroenterol* 2008; 14(33): 5154-5161

<sup>58</sup> Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased Expression of IL-17 in IBD. *Gut* 2003; 52: 65-70

<sup>59</sup> Diveu C, Lak-Hal AH, Froger J, Ravon E, Grimaud L, Barbier F, Hermann J, Gascan H, Chevalier S. Predominant Expression of the Long Isoform of GP130-Like (GPL) Receptor is Required for IL-31 Signaling. *Eur Cytokine Netw* 2004; 15: 291-302

### 1.4.5 MALT (Mucose-Associated Lymphoid Tissue)

The immune system can perform a defensive action of the body against pathogens in which it comes in contact with. This defense action takes place thanks to the synergy of the inherent components constituent of Immunity Innate and Adaptive Immunity.<sup>60</sup>

The most important defense mechanism against pathogens is put in place by the Mucosal Immune System (MALT - Mucosa-Associated Lymphoid Tissue).

The MALT, present in our intestinal mucosa, is indicated in a specific manner like GALT (Gut-Associated Lymphoid Tissue).

It is made up of specific cellular components (Peyer's Plaques) and mesenteric lymph nodes.

The immune cellular components that characterize it are represented by: T and B Lymphocytes, Dendritic cells, M cells, Paneth cells and Intraepithelial Lymphocytes.<sup>61</sup>

Their role is to ensure a full immune response both in the humoral and cell type against all antigenic exogenous and endogenous stimuli which is continually exposed to the intestinal mucosa.<sup>62</sup>

The intestinal microbiota has demonstrated the ability to interact closely with the GALT ensuring proper and effective immuno-biological modulation of the microenvironment of the intestine.<sup>63</sup>

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<sup>60</sup> Abbas, A., & Lichtman, A. Cellular and Molecular Immunology, 5th ed. (2003).

<sup>61</sup>Wershil BK, Furuta GT. Gastrointestinal mucosal immunity. J Allergy Clin Immunol 2008 Feb;121(2 Suppl): S380-3

<sup>62</sup> Jump RL, Levine AD. Mechanisms of natural tolerance in the intestine. Implications for inflammatory bowel disease. 2004; Vol. 10: pp 462–478

In particular, the resident bacterial flora in our intestines exerts its influence on the local immune system, due to its interaction with specific receptors: Pattern Recognition Receptors (PRRs).

Specific PRRs referred to as Toll Like Receptors (TLR), are localized on the surface of intestinal epithelial cells can recognize specific molecular determinants of microbial origin called Pathogen-Associated molecular Patterns (PAMPs).

Recognition of microbial antigens takes place thanks to the identification by TLR of specific components of the bacterial cell: Peptidoglycan, Lipoproteins surface, Lipoteichoic Acid and Lipopolysaccharide (LPS).

The phase following the recognition of these antigens, is characterized by the detection by TLR factors of antimicrobial actions to stop the microbial growth and the subsequent infection process that follows.

The reduced expression of TLRs in the intestine is responsible for the appearance of dismicrobism resulting in mucosal inflammation.

In fact, studies show that the quali-quantitative alteration of the intestinal bacterial flora is one of the most important trigger factors in determining a chronic overstimulation of the GALT.<sup>64</sup>

This dysregulated local immune response is characterized by the involvement of specific components (leukocytes, lymphocytes, CD4, arachidonic acid, reactive species and stress proteins) that, peppering the intestinal mucosa, are

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<sup>63</sup> Gasbarrini A, Lauritano EC, Garcovich M. New Insights into the Pathophysiology of IBS: Intestinal Microflora, Gas Production and Gut Motility. *Eur Rev Med Pharmacol Sci* 2008; 1: 111-117

<sup>64</sup> Llopis M, Antolin M, Carol M. Lactobacillus Casei Downregulates Commensals Inflammatory Signals in Crohn's Disease Mucosa. *Inflamm Bowel Dis* 2009; 15(2): 275-283

responsible for the continuous stimulation and maintenance of the chronic inflammatory process, characteristic of the UC.<sup>65</sup>

Studies also show that in these cases, the immune structure is characterized by an overexpression of the Th2 response mediated by atypical NK cells responsible for the stimulated production of pro-inflammatory specific cytokines.<sup>66</sup>

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<sup>65</sup> Arseneau KO, Cominelli F. Leukocytapheresis in Ulcerative Colitis: a Possible Alternative to Biological Therapy? *Dig and Liver Dis* 2009; 41: 551-552

<sup>66</sup> Heller F, Florian P, Bojarski C. IL-13 is the Key Effector Th2 Cytokine in Ulcerative Colitis that Affects Epithelial Tight Junctions, Apoptosis and Cell Restitution. *Gastroenterology* 2005; 129: 550-564

## 1.5 Clinical Aspects of the UC

The UC is a chronic inflammatory bowel disease with a quite heterogeneous clinical course.<sup>67</sup>

At a clinical level, patients with UC have a course of generally chronic-recurrent in which periods of acute symptoms alternate with periods of remission, with significant reduction in the accompanying clinical symptoms or even complete absence of symptoms.<sup>68</sup>

The abdominal pain, the tenesmus, the urgency and bloody diarrhea are the most common symptoms reported by the patient with UC.<sup>69</sup>

The inflammatory process that characterizes the disease has only partial involvement of the colonic wall, involving only the mucosal and submucosal layer.

The intensity and extent of inflammation allow the definition of different forms of UC. In particular, the inflammatory process can affect exclusively the rectal mucosa by determining, in this case, the onset of a typical clinical picture of proctitis or in certain cases, may extend proximally to the sigmoid colon, presenting a picture of distal colitis.

Other times, it can affect the left colon with a pattern of the left colitis or involving the entire colon with a pattern of pancolitis.<sup>70</sup>

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<sup>67</sup> Yamamoto-Furusho JK. Clinical Epidemiology of UC in Mexico: a Single Hospital-Based Study in a 20-year Period. *J Clin Gastroenterol* 2009; 43(3): 221-224

<sup>68</sup> Gurel S, Kiyici M. UC Activity Index: a Useful Prognostic Factor for Predicting UC Outcome. *The Journal of International Medical Research* 2005; 33: 103-110

<sup>69</sup> Carter MJ, Lobo J, Travis SPL. Guidelines for the Management of IBD in Adults. [www.gut.bmj.com](http://www.gut.bmj.com)

<sup>70</sup> Silverberg MS, Satsangi J, Ahmad T. Toward an Integrated Clinical, Molecular and Sierological Classification of IBD: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; 19(SA): 5-36



Recent data indicate that, at the time of diagnosis, 30-50% of patients have a distal colitis with involvement of the rectosigmoid, 20-30% have a picture of left ulcerative colitis while 20% have pancolitis.<sup>71</sup>

The degree of extension of the mucosal inflammatory process certainly represents one of the most important predictors about the course and severity of the disease.

In this regard, the data suggest that the extent of the disease is crucial in terms of colectomy, in fact, patients with pancolitis have a 3.5-4 times higher risk than in patients with proctitis.<sup>72</sup>

In relation to the degree of extent of the disease and the extent of the clinical pattern that goes with it, different forms of UC are identified: a chronic-recurrent forms, remission shapes and fulminanting forms.<sup>73</sup>

The Ulcerative Colitis Severity Index Score, an important predictor of clinical evolution of the disease, is used to distinguish a mild, moderate and severe UC.<sup>74</sup>

Recent data indicate that 64% of patients have a mild form of UC, 7% have moderate and 17% a severe form.<sup>75</sup>

The degree of severity of the disease can also be evaluated through the Mayo Score.<sup>76</sup> (Table 4)

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<sup>71</sup> Ordas I, Eckmann L, Talamini M, Baumgart DG, Sandborn JW. Ulcerative Colitis. *Lancet* 2012; 380: 1606-1609

<sup>72</sup> Hoie o, Wolters FL, Riis L, and the European Collaborative Study Group of IBD. Low Colectomy Rates in UC in an Unselected European Cohort Followed for 10 years. *Gastroenterology* 2007; 132: 507-515

<sup>73</sup> Stenson WF, Korzenik J at al. IBD. *Textbook of Gastroenterology* 2003; 4Th ED: 1699-1759

<sup>74</sup> Jun Yun, Chang-Tai XU, Bo-Rong Pan. Epidemiology and Gene Markers of UC in the Cinese. *Wolrd J Gastroenterol* 2009; 21: 15(7) 788-803

<sup>75</sup> Paiotti AP, Artigiani Neto R, Forones NM. Immuno Expression of Cyclooxygenase-1 and 2 in UC. *Brazilian J Med Biol Res* 2007; 4(7): 911-918

<sup>76</sup> Chang Hwan Choi, Sung AE Jung, BO IN LEE. Diagnostic Guideline of UC. *Korean J Gastroenterology* 2009; 53: 145-160

### Ulcerative Colitis Activity Index - Mayo Score

Score	Grade	Criteria
Stool Frequency	0	Normal number of stool
	1	1-2 stools more than normal
	2	3-4 stools more than normal
	3	>5 stools more normal
Rectal Bleeding	0	No blood seen
	1	Streaks of blood with stool less than half the time
	2	Obvious blood with stool most of the time
	3	Blood alone passed
Findings of Proctosigmoidoscopy	0	Normal or inactive disease
	1	Mild disease (erythema, decreased vascular pattern, mild friability)
	2	Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
	3	Severe disease (spontaneous bleeding, ulceration)
Physician's global assessment	0	Normal
	1	Mild
	2	Moderate
	3	Severe

Tab. 4 Texbook: G. Tomasello, P. Damiani. La Rettocolite Ulcerosa: Approccio Metodologico alla Interpretazione della Malattia e Prospettive Future. Medical Books 2010

The UC, and in general IBD, are characterized not only by a clinical pattern of intestinal involvement, but also for the appearance of symptoms related to the involvement of other organs.

For this reason, the detection of extraintestinal manifestations allows the consideration of the IBD as systemic diseases.<sup>77</sup>

The data indicate that extraintestinal manifestations may report directly to the chronic inflammatory process characteristic of the disease. And so, they are manifestations of specific diseases which require a common pathogenic mechanism with IBD.<sup>78</sup>

Other events seem, however, not directly related to intestinal disease but the genetic susceptibility for common autoimmunity.<sup>79</sup>

Published studies indicate that 6-47% of patients with UC have extraintestinal manifestations.<sup>80</sup>

These events may affect different organs and systems compromising significantly the clinical picture of IBD. (Table 5)

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<sup>77</sup> Danese S, Semeraro S, Papa A, Roberto I, Scaldaferrri F, Fedeli G, Gasbarrini A. Extraintestinal Manifestations in IBD. *World J Gastroenterol* 2005; 11(46): 7227-7236

<sup>78</sup> Das Km, Relationship of Extraintestinal Involvements in IBD: New Insights Into Autoimmune Pathogenesis. *Dig Dis Sci* 1999; 44: 1-13

<sup>79</sup> Veloso FI, Carvalho J, Magro F. Immune-Related Systemic Manifestations of IBD. A Prospective Study of 792 Patients. *J Clin Gastroenterol* 1996; 23: 29-34

<sup>80</sup> Robert C, Langan MD, Patricia B. UC: Diagnosis and Treatment. *American Family Physician* 2007; 76: 1323-1330

Extraintestinal Complications and frequency

Pericholangitis	30
Sclerosing cholangitis	1
Hepatocellular fatty infiltration	30
Chronic active hepatitis	5
Cirrhosis	3
Amyloidosis	1
Anemia	15
Iron deficiency	5
Autoimmune hemolytic anemia	1
Thrombocytosis	<1
Thromboembolic disease	20
Peripheral arthritis, migratory, nondeforming, large-joint seronegative	2
Ankylosing spondylitis, sacroilitis	20
Erythema nodosum	20
Pyoderma Gangrenosum	3
Episcleritis, uveitis	4
Iritis	5

Table 5: First Principles of Gastroenterology  
5Th Ed. Jansenn Ortho

## 1.6 The Diagnostic Path in the UC

The diagnostic uses of UC implementation of different laboratory tests, instrumental and imaging that, in a synergistic way, enable a precise classification of the disease.

Their purpose is to make a certain diagnosis of the disease, to assess their activity status, to delineate the degree of extension and severity of the inflammatory process as well as the possible presence of extraintestinal manifestations.

The Gold Standard diagnosis of the UC is surely represented by colonoscopy and biopsy with histological examination of the intestinal mucosa.<sup>81</sup>

A colonoscopy allows a direct view of the intestinal mucosa, to determine the extent of the disease and the severity of the inflammatory process based on the assessment of the Mayo Endoscopic Score. (Fig.5)

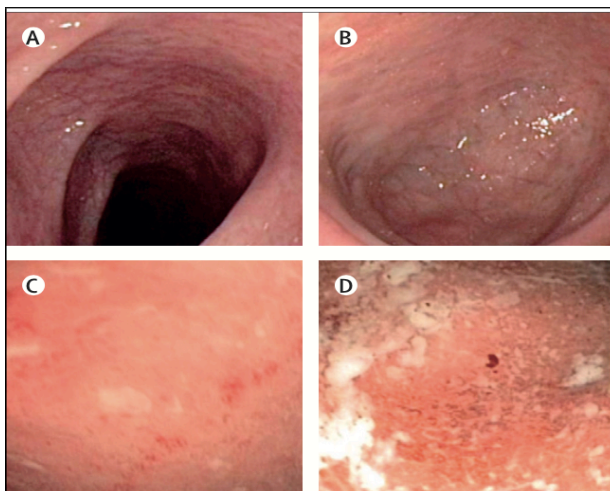


Fig.5: Mayo endoscopic score for ulcerative colitis

(A) Score 0=normal; endoscopic remission. (B) Score 1= mild; erythema, decreased vascular pattern, mild friability. (C) Score 2=moderate; marked erythema, absent vascular pattern, friability, erosions. (D) Score 3=severe; spontaneous bleeding, ulceration. Ingrid Ordás, Lars Eckmann, Mark Talamini, Daniel C Baumgart, William J Sandborn. Ulcerative colitis. Lancet 2012; 380: 1606–19

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<sup>81</sup> D'Argenio G, Cosenza V, Riegler G. Serum Transglutaminase Correlates with Endoscopic And Histopathologic Grading in Patients with Ulcerative Colitis. Dig Dis Sci 2001; 46: 649-657

Histopathologic biopsy examination of the intestinal mucosa appears to be essential for the diagnosis of certainty of UC and for a precise histological characterization of the lesions of the colonic mucosa.<sup>82</sup>

It also determines the extent of mucosal inflammatory process allowing to classify the patients according to the degree of disease activity in accordance to the Histological Activity Index (HAI). (Tab. 6)

Inflammatory Activity	Score	Histopathologic Defining Characteristics
Inactive/quiescent/normal	0	No epithelial infiltration by neutrophils
Mildly active	1	Neutrophils infiltration of <50% of sampled crypts or cross section, no ulcers or erosions
Moderately active	2	Neutrophils infiltration of >50% of sampled crypts or cross sections, no ulcers or erosions

Table 6: Histological Activity Index (HAI) – Gastroenterology 2007

The information obtained from the colonoscopy and histopathological biopsy examination helps to determine the Disease Activity Index.<sup>83</sup>

The endoscopic and histopathological survey appear to be necessary not only in the diagnostic phase but also during the follow-up to monitor response to treatment of the patient.<sup>84</sup>

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<sup>82</sup> Goetz M, Kiesslich R. Advanced Imaging of the Gastrointestinal Tract: Research vs Clinical Tools? *Curr Opin Gastroenterol* 2009; 25(5): 412-421

<sup>83</sup> Osada T, Ohkusa T, Yokoyama T. Comparison of Several Activity Indices for the Evaluation of Endoscopic Activity in Ulcerative Colitis: Inter-and Intraobserver Consistency. *IBD* 2010; 16(2): 192-197

<sup>84</sup> Manes G, Imbevi V, Ardizzone S. Appropriateness and Diagnostic Yield of Colonoscopy in the Management of Patients with Ulcerative Colitis: a Prospective Study in a Open Access Endoscopy Service. *IBD* 2008; 14(8): 1133-1138

## 1.7 The UC Therapeutic Management

The UC therapeutic management requires a long-term treatment, very often based on the use of different drugs to control the disease better.<sup>85</sup>

In fact, the therapeutic treatment arises with the potential achievement of several objectives:

1. To reduce mucosal inflammation and try to get a restitutio ad integrum of the intestinal mucosa
2. To improve the quality of patient's life by intervening on functional intestinal discomfort (abdominal colic, dysentery, abdominal bloating)
3. To reduce to a minimum the side effects and adverse reactions related to a long therapeutic treatment
4. To maintain long stages of disease remission
5. To reduce the possibility of complications of the disease

In relation to the diverse manifestation of the disease, to the seriousness and severity of the clinical pattern, to the possible presence of extraintestinal manifestations and / or complications, the management of RCU appears to be significantly diversified and is imprinted on the application of the already well established therapeutic protocols and others with a more recent formulation.

### Corticosteroids

Numerous studies confirm the effectiveness of steroids in the UC treatment in the acute stage.<sup>86</sup>

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<sup>85</sup> World Gastroenterology Organisation Global Guidelines 2009

<sup>86</sup> Richard P, MacDermott MD, Jesse A. Wath is the Optimal Therapy for severe Ulcerative Colitis? *Inflamm Bowel Dis* 2008; 14 S2

In particular, approximately 70% of patients go to clinical remission following treatment with corticosteroids even if this treatment is not particularly effective in maintaining the remission stage.<sup>87</sup>

### Acid 5-Aminosalicylic Acid (5-ASA)

The elective use of Aminosalicylates in the UC is the maintenance stage of clinical remission.<sup>88</sup>

Studies show that taking 5-ASA may reduce the risk of colorectal cancer by up to 75% and this justifies the long-term treatment of patients with UC.<sup>89</sup>

### Thiopurines

The Thiopurines are effective both for the treatment of UC in the active stage and in the maintenance stage of remission.

The indication for use of Thiopurines should include patients who undergo multiple relapse episodes of the disease during the year or a relapse within 6 weeks from the interruption of corticosteroids.<sup>90</sup>

### Methotrexate

The Methotrexate therapy is reserved for the treatment of patients with a severe and complicated form of UC with extraintestinal manifestations.<sup>91</sup>

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<sup>87</sup> Gurel S, Kiyici M. Ulcerative Colitis Activity Index: a Useful Prognostic Factor for Predicting Ulcerative Colitis Outcome. *The Journal of International Medical Res* 2005; 33: 103-110

<sup>88</sup> Sutherland LR, Roth D, Beck P. Oral 5-Aminosalicylic Acid for Maintaining Remission in Ulcerative Colitis. *Cochrane Database Syst Rev* 2002

<sup>89</sup> Eaden J, Abrams K, Ekbom A. Colorectal Cancer Prevention in Ulcerative Colitis: a Case-Control Study. *Aliment Pharmacol Ther* 2000; 14: 145-153

<sup>90</sup> McGovern DPB, Travis SPL. Thiopurine therapy: When to Start and When to Stop. *Eur J Gastroenterol Hepatol* 2003;15: 219–24

<sup>91</sup> Peluso R, Attenu M, Iervolino S. Methotrexate in the Treatment of Pheripheral Arthritis in Ulcerative Colitis. *Reum* 2009; 61(1): 15-20



## Ciclosporina

The immunosuppressive therapy with this drug allows to obtain an improvement of the mucosal inflammatory thanks to its action of inhibition of the production of pro-inflammatory cytokines and the activation of remedy processes of the intestinal epithelium.<sup>92</sup>

## Immunobiological Therapy (Infliximab)

It is an emerging therapeutic possibility that involves the use of specific monoclonal antibodies with anti-TNF-Alpha.

Its use is widely used in patients with UC in the active stage immune to steroid therapy and other conventional treatments.<sup>93</sup>

## Surgical Treatment

The surgical treatment of the UC does not surely represents the first choice treatment but it is reserved to selected patients.

Several parameters are considered during the assessment of a possible surgical treatment: high scores of Ulcerative Disease Activity Index, the extension and severity of the mucosal inflammatory process, the possible complications, the non responsiveness to conventional drug treatment and the neoplastic transformation.<sup>94</sup>

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<sup>92</sup> Satoh Y, Ishiguro Y, Sakuraba H. Cyclosporin Regulates in Intestinal Epithelial Apoptosis Via TGF-(Beta) Related Signalling. *Am J Physiol Gastrointest Liver Physiol* 2009; 297(3): 514-519

<sup>93</sup> Rutgeerts P, Sandborn WJ, Feagan BG. Infliximab for Induction and Maintenance Therapy for Ulcerative Colitis. *NE J Med* 2005; 353: 2462-2476

<sup>94</sup> Filik L, Dagli U. A Retrospective Evaluation of the History of Treated Ulcerative Colitis. *Bratisl Lek Listy* 2009; 110(4): 253-257

## 1.8 Probiotics

In years, various scientific traces have widely demonstrated the rational use of Probiotics in the prevention and treatment of numerous affection.<sup>95</sup>

The term probiotic was used for the first time at the beginning of the last century by Nobel laureate Elie Metchnikoff who, following a number of observational studies, suggested that the longevity of the Caucasian shepherds, could be due to the assumption of foods containing live lactic bacteria.<sup>96</sup>

These observations gave rise to a long series of scientific and research studies aimed to study the composition of the human intestinal microbiota and the potential use of bacterial strains with probiotic action to stimulate and secure the natural well-being of our body.

The definition of Probiotics, as provided by current guidelines published by the Ministry of Health, is the one adopted in 2001 by the Expert Consultation FAO / WHO. It defines Probiotics as "live and vital microorganisms which, when administered in adequate amounts as part of a food or a supplement, gives benefit on the human health."<sup>97</sup>

The same guidelines define Food / Supplements with Probiotics those containing micro-organisms, in sufficiently large number that can reach the intestine, multiply and exercise a beneficial effect on the human health / well-being.

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<sup>95</sup> Kaur IP, Chopra K, Saini A. Probiotics: Potentiale Pharmaceutical Applications. *Eur J Pharm Sci* 2002; 15: 1-9

<sup>96</sup> Metchnikoff E. *The Prolungation of Life*. Putman and Sons, 1907

<sup>97</sup> FAO/WHO Expert Consultation. *Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*, 2001. [www.fao.org](http://www.fao.org)

A careful examination of the international scientific literature shows a growing number of micro-organisms considered as Probiotics.

Notwithstanding, it is difficult to list thoroughly all the bacterial species in use considered as Probiotics. At present, the probiotic activity has been recognized only for specific strains. (Tab. 7)

<b>Bifidobacterium Species</b>	<b>Lactobacillus Species</b>	<b>Other Species</b>
B. Bifidum	L. Acidophilus	B. Cereus
B. Longum	L. Rhamnosus	Enterococcus Faecium
B. Breve	L. Gasseri	Enterococcus Faecalis
B. Infantis	L. Reuteri	Streptococcus Thermophilus
B. Lactis	L. Bulgaricus	Clostridium Butyricum
B. Adolescentis	L. Johnsonii	Escherichia Coli
	L. Paracasei	Saccharomyces Boulardii
	L. Casei	Saccharomyces Cerevisiae
	L. Salivarius	
	L. Lactis	

Table 7: Heyman M and Menard S. Cellular and Molecular Life Sciences.  
Vol. 59, 2002

One of the key features that a Probiotic should have is the precise identification of the bacterial strains it contains.

A careful reading of the guidelines prepared by FAO / WHO (2001- 2002), shows how this feature turns out to be absolutely necessary and that the

selection of strains to be used in Probiotic preparations must follow a meticulous process of selection to ensure the taxonomic identity, the specific phenotypic characteristics, security of use and their potential effectiveness.<sup>98</sup>

The bacterial microorganisms must meet several other requirements to be considered as Probiotics: it must be safe for human use, it must be active and vital when administered in sufficient quantity so as to carry out the beneficial effects, it must be able to reproduce as much as possible permanently in time and finally it must be able to confer a benefit to the body.

The effects of a probiotic and the therapeutic response are strictly dependent on the interaction between its metabolic functional activities and those of the host.

Studies confirm that they appear to be strain-specific.

The importance of the strain specificity is considered one of the fundamental requirements to obtain an effective therapeutic response, as documented by FAO and WHO: "data Obtained with one specific probiotic food can not be extrapolated to other foods containing that particular probiotic strain or to other probiotic microorganisms".

The AFSSA (Agence Française de Sécurité Sanitaire des Aliments) states that: "the quantity of live probiotics passing through the gut depends on the strain, the dose ingested, factors related to the host and the vector food".

Therefore, in the light of these observations, the treatment of both intestinal pathological conditions and extraintestinal bowel with probiotics, can not in any way regardless of the exact taxonomic definition and the amount of bacteria strains administered.

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<sup>98</sup> FAO/WHO Expert Consultation. Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 2001. [www.fao.org](http://www.fao.org)

In relation to the amount of bacterial strains to be administered to the patient, the document drafted by the AFSSA shows that:

- 1)“the dose of probiotics ingested is an important factor to obtain high concentrations in the various compartments of the gastrointestinal tract”;
- 2)“it is often said that probiotic concentrations must be greater than or equal to  $10^6$  CFU/ml in the small intestine (ileum) and  $10^8$  CFU/g in the colon”.<sup>99</sup>

From what can be deduced from published studies, the bacterial strains considered to be probiotics and therefore could potentially be used in humans for the treatment of specific medical conditions, must be distinguished from other bacterial strains erroneously considered probiotics and used for fermentation of dairy products .

The latter, in fact, are defined as "lactic acid bacteria", they are not human-derived and are exclusively used as food supplements.<sup>100</sup>

In the light of all the studies published up to now, it is clear that, for some specific pathological conditions, the probiotics find a real scientific rationale use while, for others, the therapeutic efficacy has not yet been fully clarified and validated.

For many years researchers have recommended the use of probiotics almost exclusively for the treatment of gastroenteritis, limiting its use in other pathological conditions, due to the limited knowledge of their functional abilities and their action mechanisms.

At present, new scientific evidence shed light on new potential ability of probiotics, expanding its range of use. Some bacterial strains have, in fact, demonstrated the ability to strictly adhere to the intestinal mucosa by antagonizing the invasion of pathogenic microorganisms not only thanks to a

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<sup>99</sup> AFSSA. Effecs Des Probiotiques et Prebiotiques sur la Flore et L'Immunitè de L'Homme Adulte. 2005

<sup>100</sup> Caramia G. Probiotics: From Metchnikoff to the Current Preventive and Therapeutic Possibilities. Med Surg Ped 2004; 26: 19-33

mechanism of territorial competition but also to the production of specific antibacterial and antifungal substances.

This way, they can correct the intestinal dismicrobism found in various pathological conditions.

Other probiotic bacterial strains possess the ability to modulate the response of our immune system by directly influencing the cytokine network.

Still others, contribute significantly to the maintenance of the physiological pattern of intestinal mucosal permeability. Lastly, it is clear that their role in the metabolic processes of proteins, carbohydrates and fats as well as in the synthesis of specific vitamins are useful for the good functioning of our body.<sup>101</sup>

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<sup>101</sup> Resta SC. Effects of Probiotics and Commensals on Intestinal Epithelial Physiology: implications for Nutrient Handling. *J Physiol* 2008; 587: 4169-4174

## 1.9 Rationale for integrated use of probiotics in the UC

In relation to etiopathogenetic factors and the pathophysiological mechanisms implicated in the onset of IBD, and in particular of the UC, over the years numerous experimental studies have been performed, both *in vitro* and *in vivo*, with the objective of verifying the existence of a potential rationale for integrated use of probiotic bacterial strains for their treatment.

The results obtained from these trials indicate that specific probiotics integration may be useful in the management of patients with UC.<sup>102</sup>

A careful analysis of the scientific literature shows that the use of probiotic preparations can be considered a potential aid in the treatment of IBD in combination with conventional therapies.<sup>103</sup>

A randomized study in particular, conducted on a sample of 120 patients with UC and treated with probiotics and symbiotics, has shown an improvement in the clinical symptoms of patients associated with a significant reduction in inflammatory markers.<sup>104</sup>

Probiotics are particularly useful in reducing the rate of recurrence after surgical treatment as well as in reducing the appearance of pauchite.<sup>105</sup>

Further study has also validated the efficacy of probiotics in the maintenance of clinical remission of inflammatory disease.<sup>106</sup>

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<sup>102</sup> Kanauchi O, Mitsuyama K, Araki Y, Andoh A. Modification of Intestinal Flora in the Treatment of Inflammatory Bowel Disease. *Curr Pharm Des* 2003; 9: 333-346

<sup>103</sup> Hedin C, Welan K, Lindsay JO. Evidence for the Use of Probiotics and Prebiotics in IBD: A Review of Clinical Trials. *Proc Nutr Soc* 2007; 66 (3): 307-315

<sup>104</sup> Fujimori S, Gudis K, Mitsu K. A Randomized Controlled Trial on the Efficacy of Symbiotic Versus Probiotic or Prebiotic Treatment to Improve the Quality of Life in Patients with Ulcerative Colitis. *Nutrition* 2009; 25 (5): 520-525

<sup>105</sup> Mach T. Clinical Usefulness of Probiotics in IBD. *Journal of Physiology* 2006; 57: (S9) 23-33

<sup>106</sup> Zocco MA, Dal Verme LZ, Cremonini F. Efficacy of Lactobacillus GG in Maintaining Remission of Ulcerative Colitis. *Aliment Pharmacol Ther* 2006; 23: 1567-1574

Other studies have confirmed that probiotics can intervene in the damage repair processes at the expense of the intestinal mucosa.<sup>107</sup>

In particular, it was shown that their repairing action of the intestinal mucosa stems from their production of SCFA.<sup>108</sup>

Probiotics also intervene, enhancing the expression of constitutive proteins of the TJ improving, in this way, the barrier function of the intestinal mucosa.<sup>109</sup>

Experimental evidence suggests a stimulation action exerted by some probiotics against goblet cell ensuring adequate production of intestinal mucus.<sup>110</sup>

In particular, it has been demonstrated that some of them stimulate the expression of genes responsible for the production of mucin (MUC2 - MUC3) resulting in an increase of approximately 60% of mucin.<sup>111</sup>

Another important rationale for the use of probiotics in the UC is their ability to correct the status of intestinal dysbiosis through several mechanisms: competition for nutrient substrates, production of substances with antibacterial and antifungal and territorial competition.<sup>112</sup>

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<sup>107</sup> Canonici C, Siret C, Pellegrino E, Pontier-Bres R et al. *Saccharomyces Boulardii* Improves Intestinal Cell Restitution Through Activation of the Alpha2 Beta1 Integrin Collagen Receptor. *Plos One* 2011; 31: 18427

<sup>108</sup> Sakata T, Kojima T, Fujieda M, Miyakozawa M, Takahashi M, Ushida K. Probiotic Preparations Dose-Dependently Increase Net Production Rates of Organic Acids and Decrease that of Ammonia by Pig Cecal Bacteria in Batch Culture *Dig Dis Sci* 1999; 44: 1485-1493

<sup>109</sup> Mennigen R, Bruewe M. Effect of Probiotics on Intestinal Barrier Function, Molecular Structure and Function of the TJ. *Ann N Y Sci* 2009; 1165, 183-189

<sup>110</sup> Mattar AF, Tehelbaum DH, Drongo Wski RA, Yongyi F, Harmon CM, Coran AG. Probiotics Up-regulate MUC-2 Mucin Gene Expression in a Caco-2 Cell-Culture Model. *Pediatr Surg Int* 2002; 18: 586-590

<sup>111</sup> Caballerp-Franco C, Keller K, De Simone C, Chadee K. The VSL3 Probiotic Formula Induces Mucin Gene Expression and Secretion in Colonic Epithelial Cells. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: 215-232

<sup>112</sup> Matur E, Eraslan E. The Impact of Probiotics on the Gastrointestinal Physiology. 2012 – [www.intechopen.com](http://www.intechopen.com)



In particular, *Saccharomyces Boulardii* has demonstrated the ability to produce specific protease capable of degrading the toxins produced by *Clostridium Difficile* and a phosphatase of 63 kDa capable of destroying the endotoxin produced by *Escherichia Coli*.<sup>113</sup>

Previous studies had demonstrated the ability of *S. Boulardii* to inhibit the growth of other pathogens: *Salmonella Typhimurium* and *Yersinia Enterocoliticum*.<sup>114</sup>

The use of probiotics in the integrated therapy of patients with UC may reduce the activity of the disease down-regulating mucosal inflammation.<sup>115</sup>

The ability of some strains of probiotics to significantly influence the cytokine network through the production of anti-inflammatory cytokines, suggested their further potential use in the UC.

The inflammatory process characteristic of IBD also stems from the activation of an important transcription factor present in the cytoplasm cell: the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-KB).

Its activation occurs following the stimulation exerted by inflammatory agents causing detachment from its precursor: IKK.<sup>116</sup>

In particular, its activation is influenced by inflammatory cytokines such as IL-1 and TNF-Alpha and endotoxin represented by lipopolysaccharides (LPS) of the cell wall of gram-bacteria.<sup>117</sup>

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<sup>113</sup> MC Farland VL. Systematic Review and Meta-Analysis of *Saccharomyces Boulardii* in Adult Patients. *World Journal of Gastroenterology* 2010; 16(18): 2202-2222

<sup>114</sup> Zibden R, Bonczi E, Altwegg M. Inhibition of *S. Boulardii* on Cell Invasion of *Salmonella Typhimurium* and *Yersinia Enterocolitica*. *Micro Ecol Health Dis* 1999; 11: 158-162

<sup>115</sup> Hanauer SB. IBD: Epidemiology, Pathogenesis and Therapeutic Opportunities. *Inflamm Bowel Dis* 2006; 12(S1): 3-9

<sup>116</sup> Neish AS, Gewirtz AT, Zeng H. Prokaryotic Regulation of Epithelial Responses by Inhibition of IKK-Alpha Ubiquitination. *Science* 2000; 289: 1560-1563

<sup>117</sup> Benelli R, Gavazzi M. La via di segnale IKK/NF-KB. *Natural Medicine* 2007

Some studies show that the initiator mechanism can stimulate the activation could be represented just by LPS and TLR expressed on the epithelial cell surface.<sup>118</sup>

Following its activation, it relocates in the cell nucleus by interacting directly with the DNA thus stimulating, the expression of specific genes.

In a specific way, it assists the expression of adhesion molecules, inflammatory cytokines, chemokines, factors growth, cyclooxygenase Cox-2 and metalloproteinases.

Recent studies identify the NF-KB as inflammatory factor common to various pathological conditions including IBD, rheumatoid arthritis, Lupus, and diabetes.<sup>119</sup>

Some probiotic bacterial strains, particularly *Lactobacillus Plantarum*, have shown to intervene on these mechanisms inhibiting the activity of NF-KB.<sup>120</sup>

The activity of NF-KB may also be inhibited by the activation of another factor: the Peroxisome Proliferator-Activated Receptor Gamma (PPAR-gamma).

The PPAR-Gamma is a nuclear receptor expressed in cells of different tissues and organs, but especially in the epithelial cells of the colon, where its expression appears to be regulated by the intestinal flora.<sup>121</sup> Its concentration appears to be significantly reduced in IBD.<sup>122</sup>

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<sup>118</sup> Chen R, Alvero AB, Silasi DA, Mor G. Inflammation, Cancer and Chemioresistance: Taking Advantage of the Toll Like Receptor Signaling Pathway. *Am J Rep Imm* 2007; 57: 93-107

<sup>119</sup> Aggarwal BB, Sethi G, Nair A, Ichikawa H. Nuclear Factor-KB: a Holy Grail in Cancer Prevention and Therapy. *Curr Signal Transduction Therapy* 2006; 1: 25-52

<sup>120</sup> Petrof EO, Claud EC, Sun J. Bacteria-Free Solution Derived from *Lactobacillus Plantarum* Inhibits Multiple NF-KB Pathways and Inhibits Proteasome Function. *Inflamm Bowel Dis* 2009; 15: 1537

<sup>121</sup> Dubuquoy L, Dharancy S, Nutten S, Auwerx J, Desreumaux P. PPAR-Gamma/RXR in IBD: a Critical Review. *Best Pract Res Clin Gastroenterol* 2003; 17: 805-820

<sup>122</sup> Dubuquoy L, Jansson EA, Deeb S. Impaired Expression of PPAR-Gamma in Ulcerative Colitis. *Gastroenterology* 2003; 124: 1265-1276

In particular, in the RCU, its reduced concentration stimulates the inflammatory process.<sup>123</sup>

The integration of specific probiotics has shown the increase, in the intestinal epithelial cells, the expression of PPAR-GAMMA inhibiting, in this way, the activation of NF-KB and significantly reducing the inflammatory process in patients with IBD.<sup>124</sup>

It was also demonstrated that the chronic inflammatory process resulting from iperexpression of TNF-ALPHA, IFN-GAMMA and other inflammatory cytokines, is able to activate specific pro-apoptotic protein.

Some probiotics inhibit their expression by adjusting, in a way, the process of cell proliferation.<sup>125</sup>

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<sup>123</sup> Bianchi Porro G, Ardizzone S, Colombo E. IBD: Year Book 2005

<sup>124</sup> Kelly D, Campbell JI, King TP. Commensal Anaerobic Gut Bacteria Attenuate Inflammation By Regulating Nuclear-Cytoplasmic Shuttling of PPAR-GAMMA and RELA. *Nut Immunol* 2004; 5: 104-112

<sup>125</sup> Yan F, Cao H, Cover TL. Soluble Proteins Produced by Probiotic Bacteria Regulate Intestinal Epithelial Cell Survival and Growth. *Gastroenterology* 2007; 132: 562-575

## 2. Aims

In the last decade, in relation to the numerous published scientific evidence, the management of UC has undergone considerable evolutions, offering a wide range of therapeutic options that are based on the application of already well established protocols and the introduction of new options of recent formulation.

The correct therapeutic strategy to be considered in the treatment of patients with UC can not be ignored in taking into account the possible and desirable goals to achieve or improve the quality and life expectancy of the patient, obtain a greater control and a possible and rapid resolution of clinical symptoms in addition to achieving a significant improvement in patient's compliance minimizing the side effects of the therapy.

The therapeutic choice should strictly depend on the history and clinical evolution of the disease in the individual patient.

Therefore, during the acute stage patients require pharmacological treatment that strictly depends on the severity of the clinical chart which, in severe cases, may require hospitalization and / or surgery.

During the remission period, the pharmacological approach aims to maintain clinical quiescence.

Careful analysis of the international scientific literature shows that the different therapeutic strategies adopted in recent years for the treatment of UC, despite offering a significant improvement in the clinical symptoms, have not showed their capacity to change radically the history of the disease in the long term clinical evolution.

In relation to numerous scientific papers published in recent years that demonstrate the potential function performed by a number of probiotic bacterial strains and the results obtained from using them, the objective of

this thesis, is to verify the effective usefulness of probiotics integration in the treatment of UC, confirming, in this way, this potential and innovative therapeutic approach to the treatment of the disease.

In the execution of this clinical study, the patients were subjected to a drug treatment of Mesalazine (Mesavancol®) and a probiotic (Acronelle®) intake, a mixture of: Lactobacillus Acidophilus, Lactobacillus Salivarius and Bifidobacterium Bifidum s.p. BGN4.

The choice of this probiotics formulation sprung from several observations on metabolic functional capabilities, already known, performed by these bacterial strains.

The choice of this probiotics formulation sprung from several observations on metabolic functional capabilities, already known, performed by these bacterial strains.

In particular, previous studies have demonstrated an important role of L. Acidophilus in IBD. Integration with Salivarius L. and B. Bifidum BGN4 has shown to boost its action.

The L. Salivarius has, in fact, demonstrated high adhesion to the intestinal epithelium providing an important trophic function and repair of the mucosa.<sup>126</sup>

The Bifidobacteria and in particular the B. Bifidum BGN4, proved to be extremely important in reducing the inflammatory processes of the intestinal mucosa, in the modulation of the immune response and in actively

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<sup>126</sup> Collins JK. Demonstration of functional properties of probiotics lactic acid bacteria (L. Salivarius). Ind. Milk 2001; 39-61 Clinical studies show the therapeutic efficacy of Lactobacillus Salivarius in IBD. (Harrison's Principles of Internal Medicine, 15th Ed, Vol 2. MC Graw Hill 2001; 1679-1695

combatting the intestinal dysbiosis detected in multiple disorders such as IBD.

Numerous studies have demonstrated that bifidobacteria has the capacity to play an important antigenotoxic role compared to the cells of the intestinal mucosa.<sup>127</sup>

The antigenotoxicity represents, in light of the extensive scientific evidence published in recent years, one of the most important potential function performed by selected probiotics bacterial strains.<sup>128</sup>

In vitro studies demonstrate the role played by bacterial pathogen overgrowth in the intestine in inducing carcinogenesis processes.<sup>129</sup>

In particular, it has been shown how the presence of Bacteroides and Clostridium overgrowth is associated with a higher incidence and speed of growth of colon cancer while on the contrary an adequate presence of Lactobacilli and Bifidobacteria results to play a protective role.

Experimental and epidemiological studies have demonstrated the ability of some strains of bacteria (bifidobacteria) to reduce the expression of several enzymes (azoreductase,  $\beta$ -glucuronidase, nitroreductase, 7- $\alpha$ -dehydroxylase) involved in the synthesis of genotoxic compounds capable of promoting the beginning of the typical carcinogenesis processes.

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<sup>127</sup> Burns AJ, Rowland IR. Anti-Carcinogenicity of Probiotics and Prebiotics. *Curr Issues Header Microbiol* 2000; 1: 13-24

<sup>128</sup> O'Mahony L, M Feeney, O'Halloran S, Murphy L, Kiely B, Fitzgibbon J, Lee G, O'Sullivan G, Shanahan F, Collins JK. Probiotic impact on microbial flora, inflammation and tumor development in IL 10 knockout mice. *Aliment Pharmacol Ther* 2001; 15 (8): 1219-1225

<sup>129</sup> Cenci G, Caldini G, F Trotta, Bosi P. In vitro inhibitory activity of probiotic spore-forming bacilli against genotoxins. *Applied Microbiology* 2008; 46 (3): 331-337

Recent studies show that *B. Bifidum* BGN4 can perform antigenotoxic action, anti-inflammatory and immune-modulator thanks to its ability to produce a complex polysaccharide (BB-Pol) consisting of Chiroinositolo, Rhamnose, Glucose, Galactose and Ribose.

The BB-pol produced by *B. bifidum* BGN4 has shown to inhibit in vitro, the growth of specific tumor cell lines of human colon.

### 3. Materials and Methods

For our study which lasted for two years (2011-2013), we recruited 60 patients with UC at the Clinic of Digestive Endoscopy of AOUP “P. Giaccone” in Palermo, and were divided into two groups of 30 elements each. Group A included 22 men and 8 women aged between 35 and 69 (average: 43) with an index of activity (Mayo Endoscopic Scoring System) between 2 and 3 (moderate-to-severe disease), treated pharmacologically with once-daily dose of 1200 mg of oral mesalazine (Mesavancol® 1200 mg cpr, Giuliani spa, Milan). 19 men and 11 women suffering from UC aged between 28 and 71 (average: 46) were included in group B and activity index always lies between 2 and 3 (moderate-to-severe disease), treated again with 1,200 mg of mesalazine per os (Mesavancol® 1200 mg cpr, Giuliani spa, Milan), this time assisted in taking daily double administration of a probiotic blend Lactobacillus salivarius and Lactobacillus acidophilus based, specific strain of Bifido bifidum BGN4 (Acronelle®, Bromatech srl, Milan, Italy).

A series of endoscopic surveillance during the first visit (t0) were programmed for each group and later 6 (t1), 12 (t2), 18 (t3) and 24 (t4) months. A new reassessment was carried out for every check-ups in accordance with Mayo endoscopic score for ulcerative colitis.

The results were used to build a database on excel sheet and the data were evaluated statistically using the ANOVA method for repeated measures (Repeated Measures ANOVA), while the two groups were compared using the t test (unpaired t test).



## 4. Results

Analysis of the results (and subsequent follow-up t0 t1-t4 - range: 24 months) shows that patients treated with mesalazine and probiotic blend had a better clinic-endoscopic score (Mayo) than patients treated with mesalazine alone (Fig. 6-20).

In particular, Figure 16 suggests that the benefits of probiotic blend in combination with the anti-inflammatory are more obvious and significant after a period of at least 18 months.

We can see from the graphs that both considered treatments have some effectiveness or otherwise provide a distinct advantage to patients with UC who use it. Between the two groups, however, the one that gets the most benefit is definitely group B, in which of course, the use of probiotic blend enhances the effects which are already positive in the anti-inflammatory.

Considering the individual parameters which are taken into account to calculate the Mayo Score, it is enough to look at figures 6 and 7 to understand out how mesalazine has positive effects in both groups, but these are already significant as early as 6 months of treatment for only group B, the one treated with the addition of the probiotic blend.

We can also see from the figures how, in reality, the effect of therapy is much more constant in B, where the values used in assessing the overall clinical condition remain consistently low, while there is a slight deterioration in A.

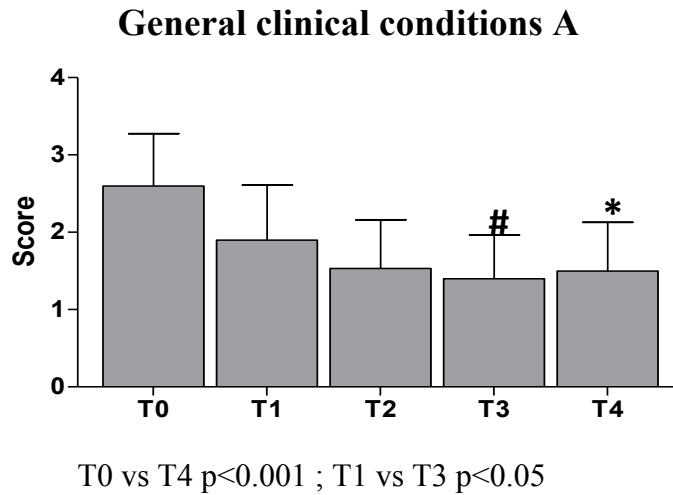
Figure 4 corroborates as specified, showing a slight benefit of the combination therapy compared with the sole use of mesalazine, with a statistically significant benefit after only 2 years of treatment.

The also frequency shows a significant reduction, this time in both groups, although it is more pronounced in the second (Fig. 9-10). It is strangely interesting to note that there is a slight deterioration at 18 months in group B,

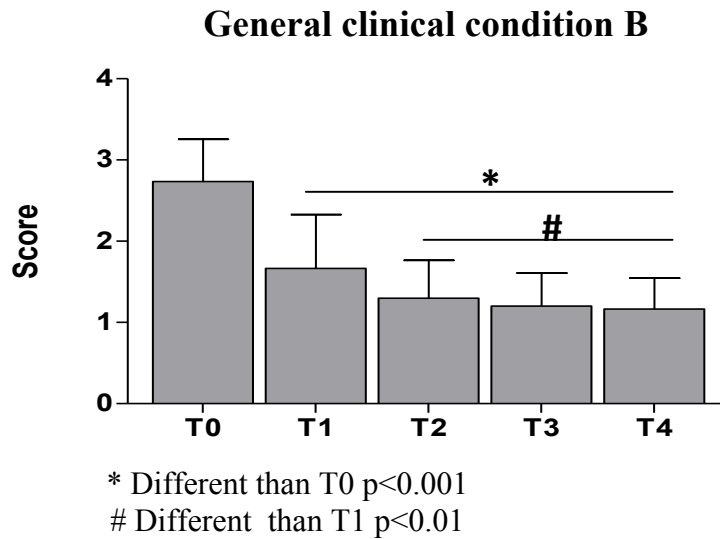
while in group A, even in this case, is encumbered by a moderate loss of effectiveness of anti-inflammatory therapy. Comparing the two groups (Fig.11), the reduction of alvo frequency becomes statistically significant at 6 and 24 months always in favor of group B. Even in this case it detects a small reversal of the trend at 18 months when the patients of group B seem to lose the advantage supplied by the probiotics.

Regarding the endoscopic picture (Fig. 12-13-14), both treatments are already valid after 6 months. In group B, however, in addition to showing greater improvement of the intestinal mucosa conditions studied, it is noted, as for the overall clinical condition, a prolonged beneficial effects of combination therapy for up to two years from the beginning of the treatment, that also in this case, does not occur for the group, which, indeed, loses some of the benefits of the therapy already at t3.

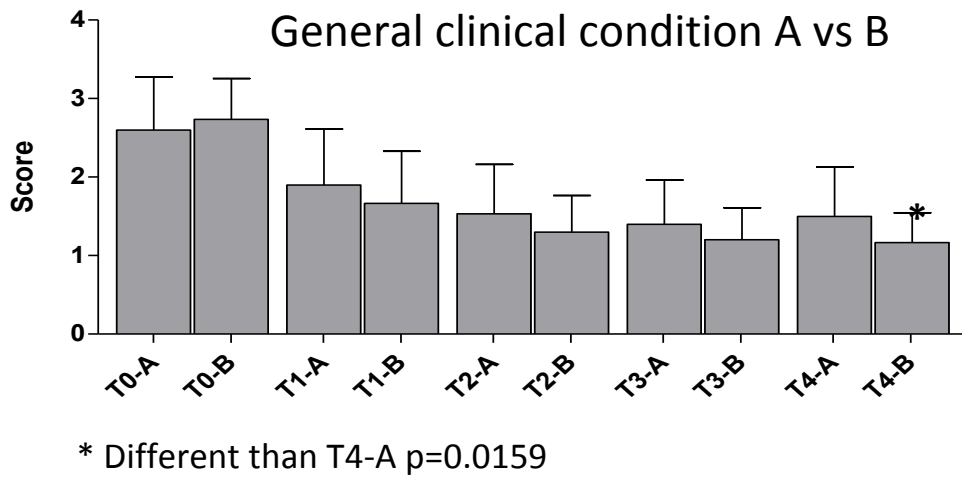
Finally, as for the endoscopic picture, the evaluation of rectal bleeding more or less follows the same time lapse (Fig. 15-16-17). In fact, it is noted that there is a minor bleeding in both groups, already at t1, but while group B enjoys the positive effects of probiotics up to 2 years, showing a certain constancy of values at the time of check-up, group A, once again, gets progressively worse from 18 months of treatment onwards.



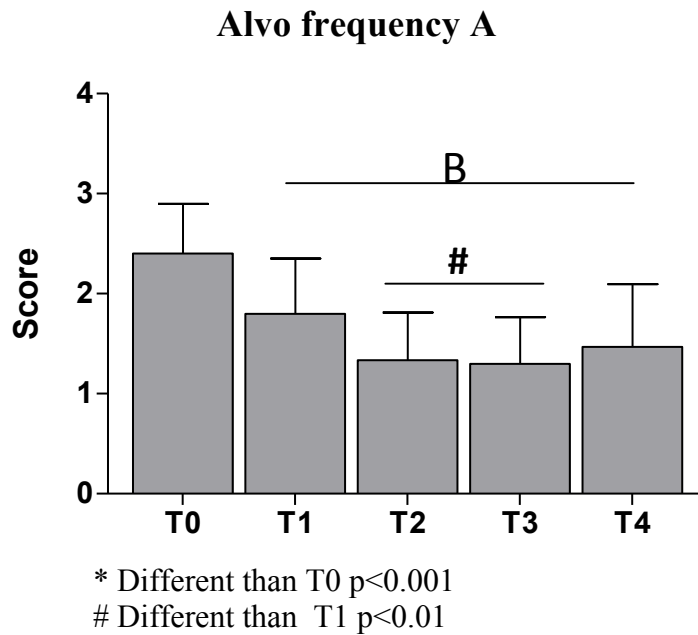
**Fig. 6 Statistical analysis of the "general clinical condition" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group A.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) in patients with moderate and severe UC at t0, implies an improvement in overall clinical condition statistically significant at 18 and 24 months (Repeated Measures ANOVA).



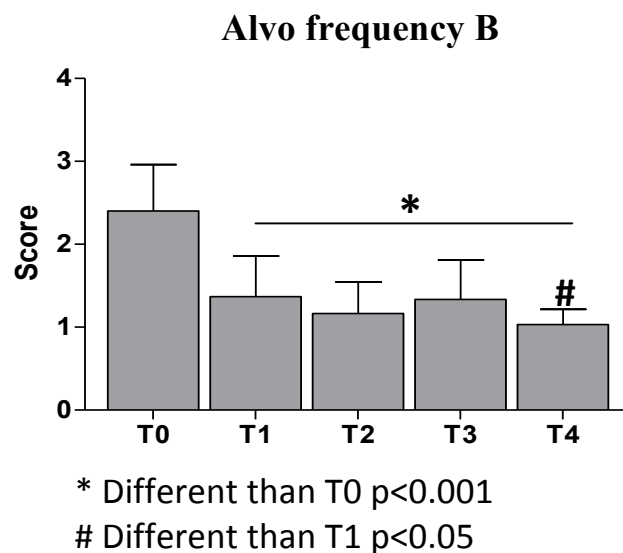
**Fig. 7: Statistical analysis of the "general medical condition" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group B.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) and the probiotic mixture (Acronelle®, Bromatech srl, Milan, Italy) in patients with moderate and severe UC at t0, implies an improvement in overall clinical condition already statistically significant after 6 months of treatment (Repeated Measures ANOVA).



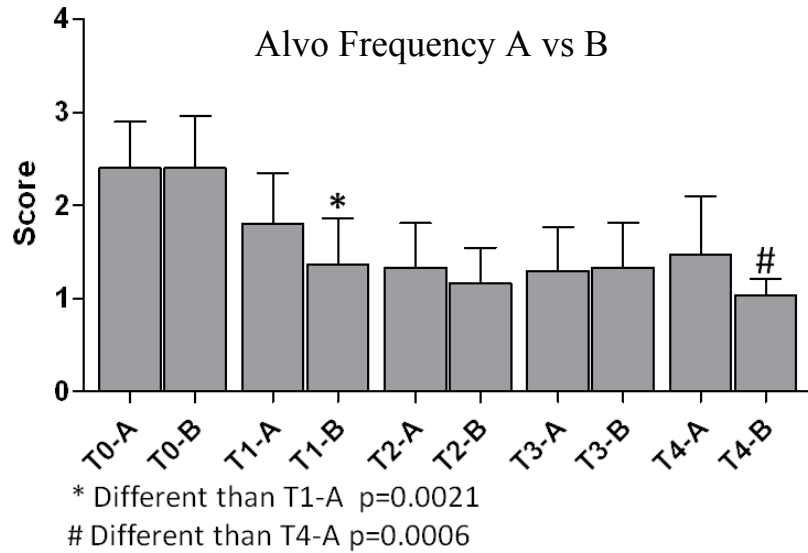
**Fig. 8: Comparison statistics between group A and group B of "general medical condition" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC).** In patients with moderate and severe UC at t0, group B shows a significant improvement in overall clinical condition compared to group A, after 2 years of treatment (Repeated Measures ANOVA).



**Fig. 9: Statistical analysis of the "alvo frequency " parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group A.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) in patients with moderate and severe UC at t0, leads to a significant reduction in the frequency of the alvo after 6 months of treatment, with a statistically higher significance at t2 and t3 (Repeated Measures ANOVA).

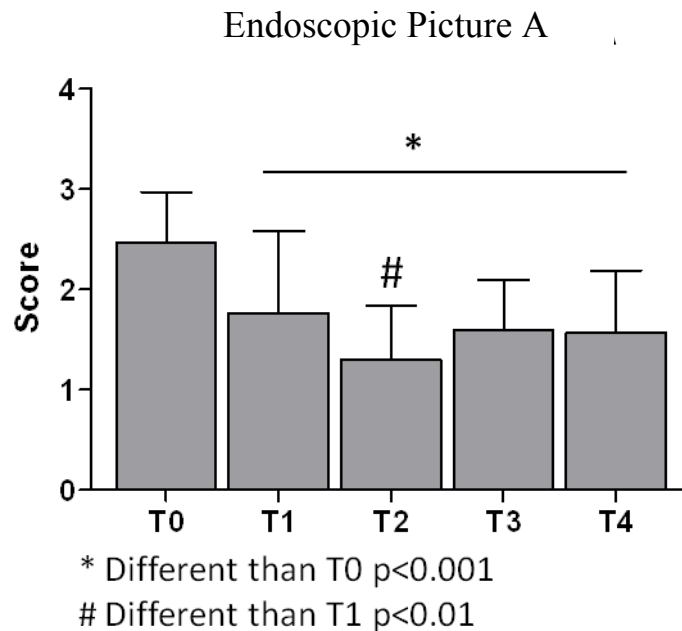


**Fig. 10: Statistical analysis of the "alvo frequency " parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group B.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) and probiotic blend (Acronelle®, Bromatech srl, Milan, Italy) in patients with moderate and severe UC at t0, leads to a significant reduction in the frequency of the alvo after 6 months of treatment, with a statistically significant increase after 2 years of initiation of treatment (Repeated Measures ANOVA).

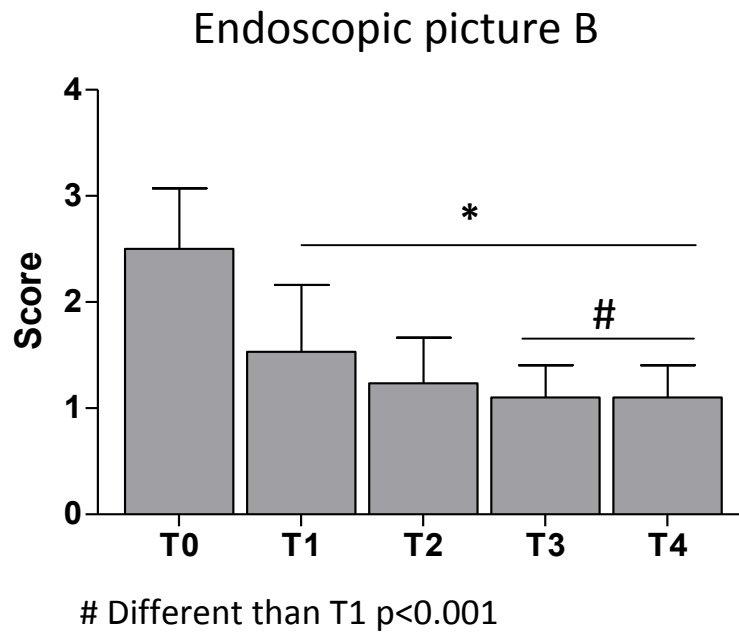


**Fig. 11: Comparison statistics between group A and group B of the "alvo frequency" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC).** In patients with moderate and severe UC at t0, group B shows a significant reduction in the frequency of the alvo, compared with group A, at 6 months and 2 years after initiation of treatment (Repeated Measures ANOVA).

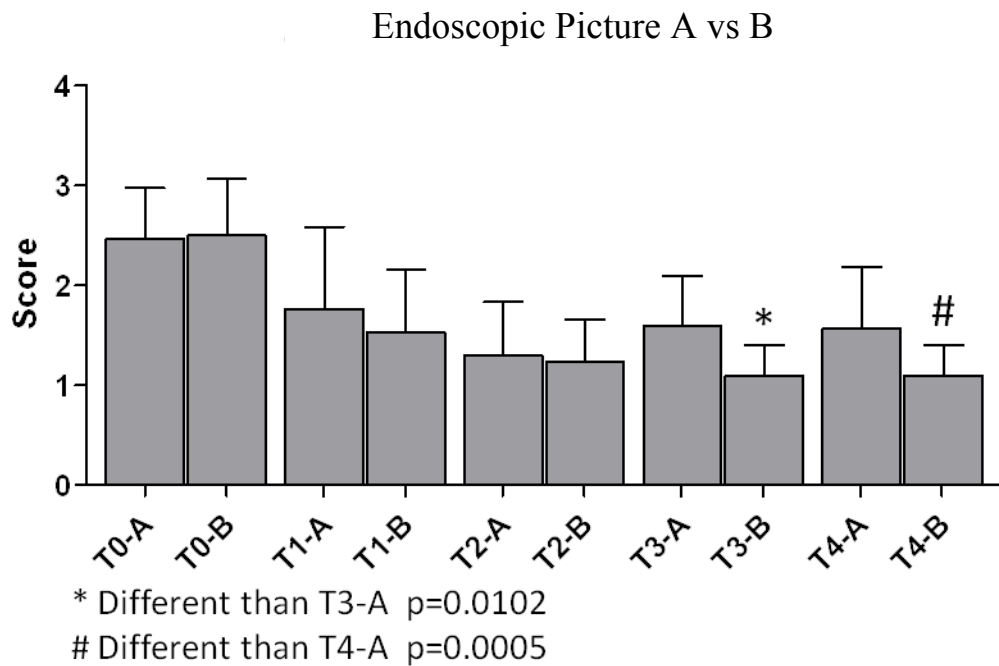




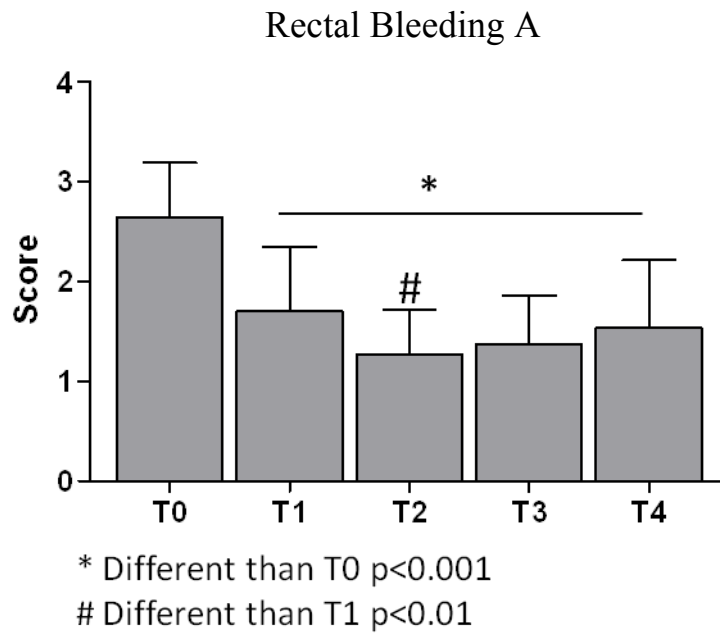
**Fig. 12: Statistical analysis of the "endoscopic picture" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group A.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) in patients with moderate and severe UC at t0, implies a statistically significant improvement in endoscopic picture after only 6 months of treatment, with greater statistical significance than t2 (Repeated Measures ANOVA).



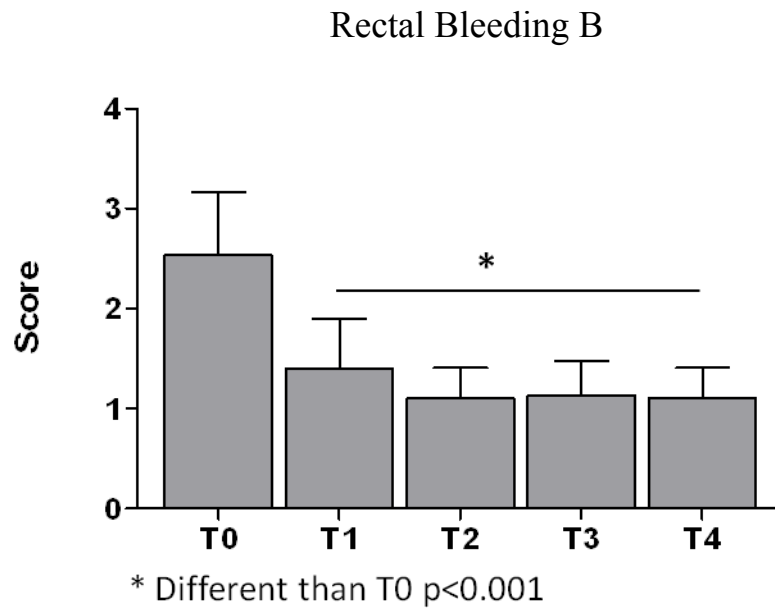
**Fig. 13: Statistical analysis of the "endoscopic picture" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group B.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) and the probiotic blend (Acronelle®, Bromatech srl, Milan, Italy) in patients with moderate and severe UC at t0, leads to a statistically significant improvement in endoscopic picture after 6 months of treatment, with even greater significance at 18 and 24 months (Repeated Measures ANOVA).



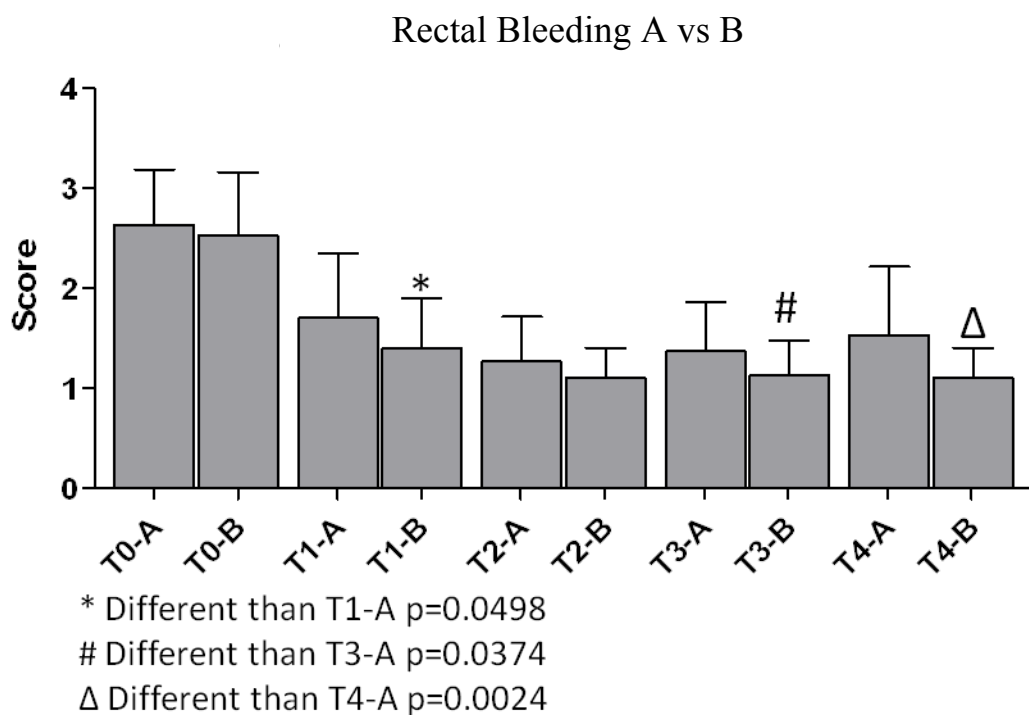
**Fig. 14: Statistical Comparison between group A and group B of the "endoscopic picture" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC).** In patients with moderate and severe UC at t0, group B shows a significant improvement in the endoscopic picture, compared to group A, starting from t3 (Repeated Measures ANOVA).



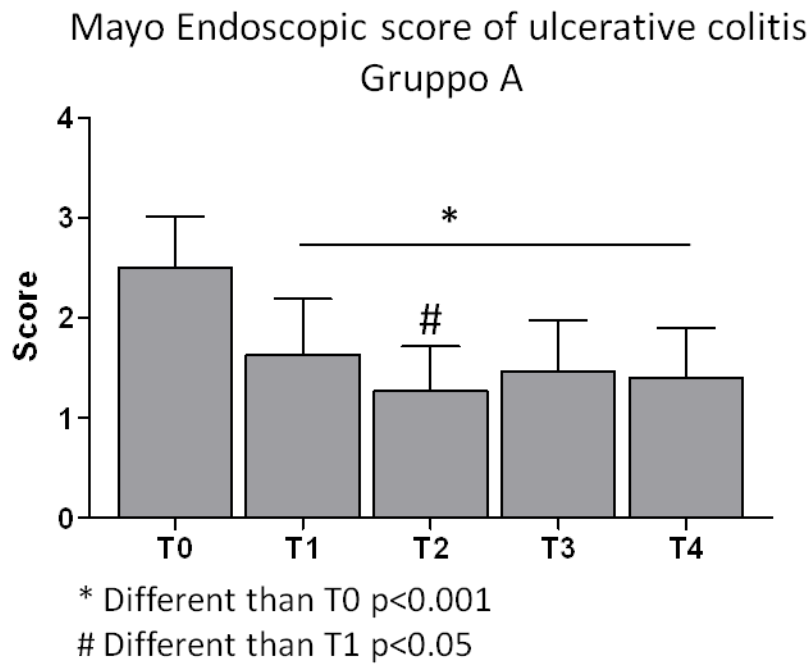
**Fig. 15: Statistical analysis of the "rectal bleeding" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group A.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) in patients with moderate and severe UC at t0, leads to a statistically significant reduction in rectal bleeding after only 6 months of treatment, with greater statistical significance than t2 (Repeated Measures ANOVA).



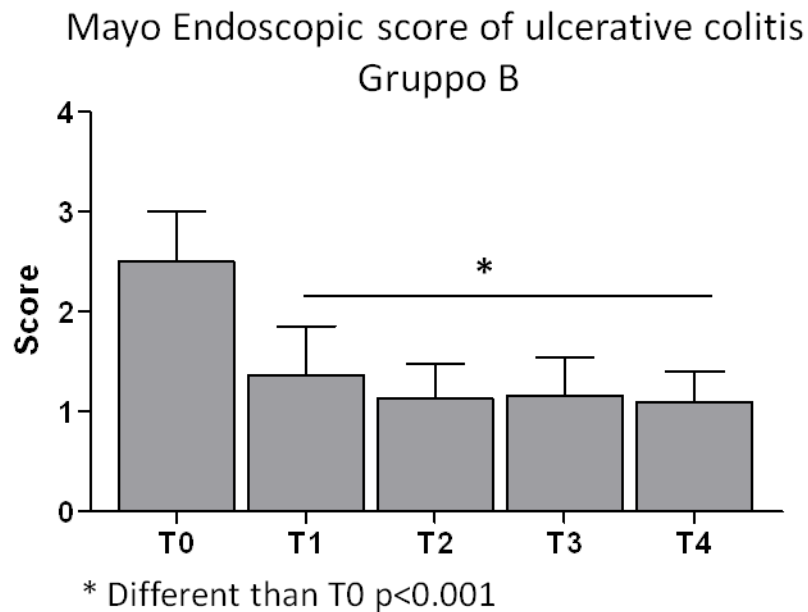
**Fig. 16: Statistical analysis of the "rectal bleeding" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC) for group B.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) and the probiotic blend (Acronelle®, Bromatech srl, Milan, Italy) in patients with moderate and severe UC at t0, leads to a statistically significant reduction in rectal bleeding after only 6 months of treatment (Repeated Measures ANOVA).



**Fig. 17: Comparison statistics between group A and group B of the "rectal bleeding" parameter of endoscopic Mayo Score for Ulcerative Colitis (UC).** In patients with moderate and severe UC at t0, group B shows a significant reduction in rectal bleeding after only 6 months of treatment compared to group A and then at t3 and t4, losing significance only at 1 year of treatment.

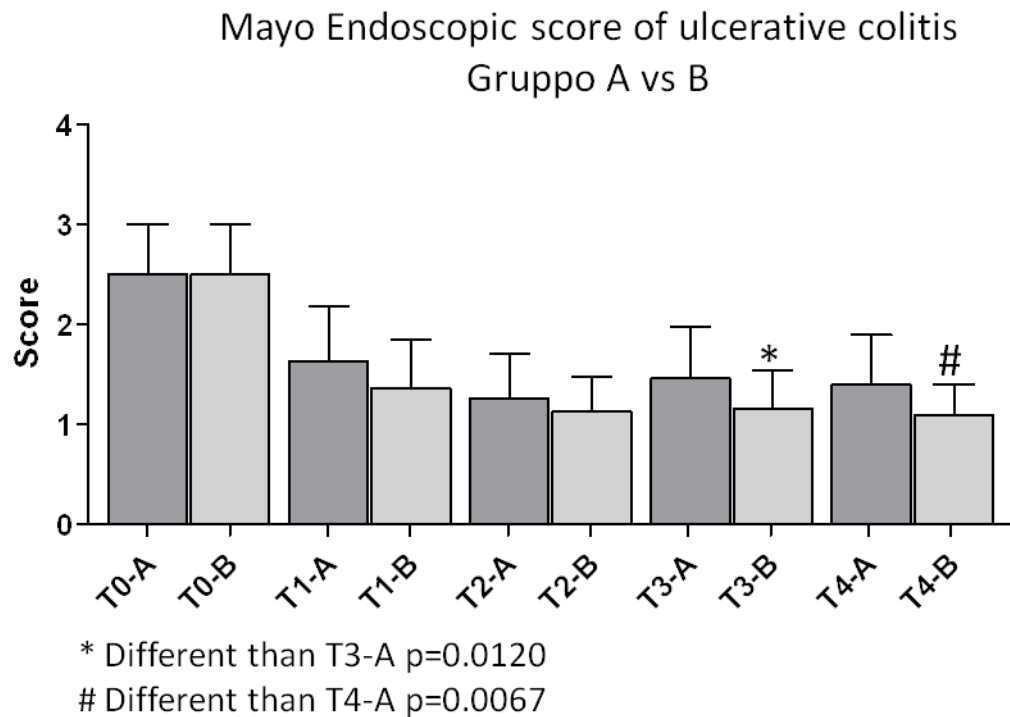


**Fig. 18: Statistical analysis of changes in the endoscopic Mayo Score for Ulcerative Colitis (UC) over time (t0-t4) relating to group A.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) in patients with moderate and severe UC at t0, leads to a statistically significant reduction of the score after only 6 months of treatment, with greater statistical significance than t2 (Repeated Measures).



**Fig. 19: Statistical analysis of changes in the endoscopic Mayo Score for Ulcerative Colitis (UC) over time (t0-t4) relating to group B.** The use of mesalazine (1200 mg cpr Mesavancol® rp, Giuliani SpA, Milan, Italy) and probiotic blend (Acronelle®, Bromatech srl, Milan, Italy) in patients with moderate and severe UC at t0, leads to a statistically significant reduction of the score after only 6 months of treatment, which remains constant for the remaining period of observation (Repeated Measures ANOVA).





**Fig. 20: Comparison of the statistical variations of endoscopic Mayo Score for Ulcerative Colitis (UC) between group A and group B.** In patients with moderate and severe UC at t0, group B shows a greater reduction of the score after only 6 months of treatment, compared to group A. But the benefits of combination therapy probiotics + mesalazine become statistically significant at 18 and 24 months of treatment (Repeated Measures ANOVA).

## 5. Discussion

The UC as the CD are considered multifactor etiopathogenesis pathologies.

The scientific literature data identify the presence of a genotype susceptibility which, together with various environmental factors, are capable of interacting with the immune system to give rise to a chronic inflammation to significantly damage the intestinal mucosa of these patients.

Although the possible genetic alterations responsible for providing for UC have been identified, the role played by environmental factors in the pathogenesis of the disease should not be underestimated.

Among the various epigenetic factors identified so far, the intestinal microbiota seems to represent one of the most important factors.

The characteristic recurrent chronic trend of the UC has led to the hypothesis that the inflammation process of the intestinal mucosa is due to the constant exposure of the mucosa to antigenic endoluminal stimuli that can stimulate the activation of an exaggerated local immune response.

Numerous studies published over the years have identified in the intestinal dismicrobism the most important endoluminal antigenic stimulus that, along with the overthrow of the whole intestinal microenvironment, can trigger the inflammatory process and hyperactivation of the immune system typical of the UC.

It is also observed in the course of IBD and in particular of UC, in addition to a state of intestinal dysbiosis, a modification of other components of the mucosa.

In particular, we can see a significant reduction in the thickness of the mucus layer associated with a reduced representation of goblet cells in addition to a

reduced synthesis of mucin resulting in the impairment of the barrier function carried out by the intestinal mucosa.

In IBD, particularly in the UC it is found, in fact, the reduced expression of specific genes responsible for the more or less adequate expression of the goblet cells.

The mucin deficiency appears to be related to the onset of intestinal dysbiosis and increased tendency to colitis.

Many studies confirm the importance of a proper production of mucous and a rich amount of mucin to avoid the onset of intestinal mucosal inflammation. Studies also show a modification of the mucous system. In fact, it is observed in patients with UC an increased intestinal permeability due to the destruction of the Tight Junction.

In fact, it is detected in UC a reduced transcription of specific gene sequences assigned to the synthesis of the protein constituent of the TJ.

The alterations of the defense system of the intestinal mucous are directly attributable to the dysregulated expression of specific cytokines inflammatory. Other than these specific alterations, a change is detected in both quality and quantity of the normal intestinal flora bacteria.

Recent studies show, in fact, that the state of intestinal dysbiosis, typical of patients with UC, is characterized by enterotype alteration with the establishment of a specific and characteristic enterotype of the IBD.<sup>130</sup>

This specific enterotype seems to be directly related to the degree of inflammation of the intestinal mucous and the extent of the clinical-symptomatic chart.

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<sup>130</sup> Hildebrand F, Brinkman B, Yunta RG, Cauwe B, Vandenabeele P, Liston A, Raes J. Inflammation-Associated Enterotypes, Host Genotype, Cage and Inter-Individual Effects Drive Gut Microbiota Variation in Common Laboratory Mice. *Genome Biology* 2013; 14: R4

In particular, the establishment of this specific enterotype results to be positively correlated with a high concentration of Fecal Calprotectin, known index of the intestinal mucous found in high concentrations in patients with UC.

Microbiological studies detect, in particular, a significant reduction of Bifidobacteria and Lactobacilli and the growth of bacterial pathogens responsible not only for the onset of persistent intestinal infection but also of micro-lesions of the mucous and of the continuous characteristic inflammatory process.

In fact, it is demonstrated in patients with IBD, the overgrowth of specific pathogenic strains as some members of the Deltaproteobacteria phylum like *Bilophila Wadsworthia*.

It is a gram-negative bacteria resistant to bile hardly found as a intestinal commensal particularly abundant in healthy subjects and in patients with IBD and responsible to subvert immune homeostasis resulting in the appearance of a low-grade mucosal inflammatory process with increase in INF-G, IL-12 and reduced IL-6, 17 and 23.

*Bilophila Wadsworthia* has shown to metabolize Sulfates producing Hydrogen Sulfide responsible for lesions of the intestinal mucous and to inhibit the production of SCFA (A. Butyric) resulting in inhibition of the processes of repair of the intestinal mucous.<sup>131</sup>

The alteration of the normal intestinal flora seems to be responsible to participate in the dysregulated immune response characteristic of local IBD.

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<sup>131</sup> Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10<sup>-/-</sup>* mice. Suzanne Devkota, Yunwei Wang, Mark W. Musch, Vanessa Leone, Hannah Fehlner-Peach, Anuradha Nadimpalli, Dionysios A. Antonopoulos, Bana Jabri & Eugene B. Chang  
Nature 487, 104–108

Studies show that micro-intestinal mucous mostly involved in the inflammation are those on which there is an increased bacterial pathogens.

Histological analysis and biopsy cultivation of the patients' mucous with UC detect a significant reduction of Bifidobacteria and lactobacilli and an increase in the concentration of pathogenic bacteria capable of inducing a pro-inflammatory effect.

The persistent intestinal dysbiosis detectable in the UC is responsible for the activation of the GALT directly connected on the onset of the inflammatory process.

Contextually, there is an alteration in the immune-tolerance biological mucous capable in inducing significant modifications of the normal resident bacterial flora.

The result of these processes is the appearance of chronic inflammation and persistent intestinal dismicrobism.

The studies show that the persistent intestinal disbiosis subtends, at least in the 70% of the patients to a reduced or non expression of the TSLP (Thymic Stromal Lymphopoietin) which is defined as "protein keeper" of our intestine, responsible for the discriminating action between beneficial resident flora and pathogen flora associated with reduced expression of the Difensins, protein responsible for the destruction of the pathogen bacteria present in our intestine.<sup>132</sup>

The clinical experience gained over the years has made it possible to identify different therapeutic protocols for the treatment of UC but none of them has proven so far to be able to significantly change the natural history of the disease.

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<sup>132</sup> Allakhverdi, Z., M. R. Comeau, et al. "Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells." *J Exp Med* 2007. 204(2): 253-8.

Studies have shown great efficacy of anti-inflammatory drugs such as 5-aminosalicylic acid and corticosteroids, but these are not always sufficient in controlling the clinical course of the disease.<sup>133</sup>

In recent years a widespread use immunosuppressive drugs have been found. These biological agents potentially capable of blocking the biochemical events which support a periodic inflammatory process characteristic of the intestinal mucosa of UC but these drugs do not always guarantee a satisfactory therapeutic result.<sup>134</sup>

These observations have prompted research to identify new therapeutic treatments that allow to act on the pathophysiological mechanisms characteristic of UC and capable of improving the quality and life expectancy of patients, to reduce significantly the clinical symptoms and to reduce to the minimum side effects.

In relation to the extensive scientific evidence that over the years have amply demonstrated and confirmed the efficacy of probiotics in the treatment of various diseases, it has been suggested its potential use in the treatment integrated with IBD, and particularly in the UC .

The use of probiotics in the clinical setting is now widely documented in both therapeutic and preventive setting.

Their potential rationale use stems from a number of studies that over the years have demonstrated the specific metabolic functional potentials they completed.

In particular, it has been repeatedly demonstrated and confirmed their ability to counteract the state of intestinal dysbiosis significantly reducing the presence of pathogenic bacteria and increasing the concentration of Bifidobacteria and Lactobacilli constituent of a normal intestinal bacterial flora.

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<sup>133</sup> Robert C, Langan MD, Patricia B. UC: diagnosis and treatment. (AAFP) American Family Physician 2007; 76: 1323-1330

<sup>134</sup> Richard P, MacDermott MD, Jesse A. What is the optimal therapy for UC? Inflamm Bowel Dis 2008. Vol 14(S2)

Specific probiotic bacterial strains such as *Enterococcus Faecium* and *S. Boulardii* are, in fact, capable of producing substances with antibacterial and antifungal which reduces the presence of *E. Coli*, of *Clostridium D.*, *Candida Albicans*.<sup>135</sup>

Other probiotics have demonstrated an intervention in the processes of production of intestinal mucus, to produce SCFA such as acetic, propionic and butyric with the capacity to nourish the intestinal mucosa and restore the barrier functions.<sup>136</sup>

Numerous studies have also demonstrated the ability of specific probiotics to go unto the cytokine network reducing mucosal inflammation process and modulating the local immune response.

A careful analysis of the scientific literature shows that the use of probiotic preparations, can be considered a potential aid in the treatment of IBD in combination with conventional therapies.

A randomized study, conducted on a sample of 120 patients with UC and treated with probiotic and symbiotic, showed an improvement of the clinical symptoms of patients associated with a significant reduction in inflammatory markers.<sup>137</sup>

Probiotics are found particularly useful in reducing the rate of recurrence after surgical treatment and to reduce the appearance of pauchite.<sup>138</sup>

A further study also validated the efficacy of probiotics in maintaining clinical remission of inflammatory disease.<sup>139</sup>

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<sup>135</sup> Borgia M, Sepe N, Brancato V. A controlled clinical study on streptococcus faecium preparation for the prevention of side reactions during long term antibiotic treatments. *Curr Ther Res* 1982; 31(2)

<sup>136</sup> Collins JK. Demonstration of functional properties of probiotics lactic acid bacteria. *Ind Latte* 2010; 39-61

<sup>137</sup> Fujimori S, Gudis K, Mitsu K. A randomized controlled trial on the efficacy of symbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with UC. *Nutrition* 2009; 25(5): 520-525

<sup>138</sup> Mach T. Clinical Usefulness of probiotics in inflammatory bowel diseases. *Journal of Physiology* 2006; 57(S9): 23-33

<sup>139</sup> Schwartz M, Cohen R. Optimizing conventional therapy for inflammatory bowel disease. *Curr Gastroenterol Rep.* 2008; 10(6): 585-590

Other studies have confirmed that probiotics are able to intervene in the process of repairing the damage to the intestinal mucosa.<sup>140</sup>

In particular, it was shown that their repairing action of the intestinal mucosa stems from their production of SCFA.

In light of these observations, the origin of this thesis, it was decided to use a specific probiotic, Acronelle (Acronelle®) containing *L. Salivarius* and *Bifidobacterium Bifidum BGN4*, in the integrated treatment of patients with UC and treated with mesalazine (Mesavancol®) with the objective of verifying the potential benefits achieved on the clinical symptoms.

*Lactobacillus Salivarius* showed to be capable of producing peptides and other antimicrobial agents with high contrast activity against numerous intestinal pathogens.<sup>141</sup>

It has a very high adhesive intestinal epithelium with respect to which it exerts trophic, restorative and anti-inflammatory functions.

The *Bifidobacterium bifidum BGN 4* is able to perform in a synergetic manner with *L. Salivarius* anti-inflammatory action against the intestinal mucosa thanks to the production of a polysaccharide (BB-Pol) containing Chiroinositolo capable of reducing the production of cytokines acting as proinflammatory and increasing the anti-inflammatory action.<sup>142</sup>

The conclusions of this study seem to confirm the initial hypothesis of a potential rationale for the use of probiotics in the management of UC and that

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<sup>140</sup> Tomasello G, Damiani P, Novi L. Intestinal bacteria and bowel disease: role of probiotics. *Capsula Eburnea* 2010; 5(20): 116-119

<sup>141</sup> Collins JK, Demonstration of functional properties of probiotics lactic acid bacteria (*L. Salivarius*). *Ind. Latte* 2001; XXXVII(1-2): 39-61

<sup>142</sup> Hyun Ju You, Deok-Kun Oh, Geun Eog Ji. Anticancerogenic effect of a novel chiroinositol-containing polysaccharide from *Bifidobacterium bifidum BGN4*. *FEMS Microbiology Letters* 240 (2004) 131–136



the combined treatment with them is significantly beneficial for patients suffering from the disease.

In particular, considering the individual parameters on the basis of Mayo Score, it shows that combination treatment in patients with moderate and severe UC involves:

- an improvement in the general clinical conditions already statistically significant after 6 months of treatment with a neat improvement at 18 and 24 months;
- an already significant reduction in the alvus frequency after 6 months of treatment, with a greater statistical significance at 18 and 24 months;
- an already statistically significant improvement in endoscopic chart after 6 months of treatment, with a greater statistical significance at 18 months and 24 months;
- an already statistically significant decrease of rectal bleeding after 6 months of treatment, with a greater statistical significance at 18 months
- a statistically significant reduction of the score as early as 6 months of treatment, with a greater statistical significance at 18 months;

The results of the study therefore show, a significant benefit of the treatment of the the UC with integrated probiotics confirming, in this way, the results published from previous scientific works which justify the rationale for the use of specific probiotic bacterial strains in the management of patients with UC.

The encouraging results obtained seem to justify further studies which would increase the knowledge about the genotypic and phenotypic characteristics of our intestinal microbiota and specific bacterial probiotics capable of

enhancing their integrated use not only in IBD but also in other pathological conditions.