



Research Article

Intestinal dysbiosis: definition, clinical implications, and proposed treatment protocol (Perrotta Protocol for Clinical Management of Intestinal Dysbiosis, PID) for the management and resolution of persistent or chronic dysbiosis

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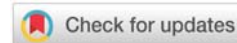
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Abstract

The human intestinal Microbiota is considered the second brain, because of its implications and correlations in hundreds of functional and dysfunctional processes. Therefore, if knowing the Microbiome (and the Microbiota) is important from a neurobioimmunological point of view, on the other hand it is equally important to investigate the correlations between dysbiosis and the onset of specific physical and psychological diseases. This study focuses on the scientific literature on natural and integrative treatments for intestinal dysbiosis, in order to identify a protocol that has the lowest possible risk and the best possible results, both in the acute phase (for the resolution of symptoms or as a preventive function) and in the chronic phase (for the management of the morbid condition and its clinical consequences).

Contents of the manuscript

Introduction

The term “intestinal microbiota” refers to the set of symbiotic microorganisms (bacteria, viruses, mycetes and protozoa) found in the human digestive tract, and is formed by different ecological niches that host a population consisting of a plurality of species and many strains; from the term “microbiota” we can distinguish the term “microbiome” which is used to refer to the totality of the genetic heritage that the microbiota is able to express. It is no coincidence that the “microbiota” is considered a real “organ within the organ”

as it performs functions that we would otherwise not be able to perform, including the ability to assimilate indigestible components of our diet, such as plant polysaccharides. The intestinal mucosa, after the respiratory one, represents the largest surface of our organism: a real defense organ that acts as a barrier against immunogenic or harmful factors present in the intestinal lumen. In fact, we coexist with many different species of bacteria; in particular, in humans there are up to a thousand different species of microorganisms (of which four hundred are just bacteria). However, the types of bacteria are different depending on the portion of the gastrointestinal tract taken into consideration, since in the stomach *Helicobacter Pylori* prevails, while in the intestine (from ileum to colon) the

bacterial species are much greater and variable. Components of the human microbiota include those that cause fermentation (80%) such as *Lactobacillus* and *Bifidobacteria*, and those that instead cause putrefaction of the remains (20%) such as *Escherichia*, *Bacteroides*, *Eubacteria* and *Clostridium*. Many are useful and harmless as constituents of the human microbiota in eubiotic equilibrium, but taken individually they can be dangerous or even deadly. Generally, these bacteria are divided into: a) commensal or physiological, which belong to the organism; b) pathogenic, which cause a disease; c) probiotic, which influence the host by improving the intestinal microbial balance. Equally important in the field of Microbiota are prebiotics, i.e. non-digestible food ingredients that in the large intestine stimulate the growth/metabolic activity of a limited number of microbial groups, important for a good functioning of the organism and symbiotics that are a combination of probiotics and prebiotics [1].

Intestinal homeostasis and intestinal dysbiosis

The intestinal homeostasis (or eubiosis), is “the natural tendency to achieve a relative stability, both of internal chemical-physical properties and behavioral, which is common to all living organisms, for which this dynamic regime must be maintained over time, even when external conditions vary, through precise self-regulatory mechanisms”. The purpose of the intestinal microbiota is to maintain this balance, as it regulates the integrity of the epithelium, the motility of the intestine (peristalsis) and the formation of the immune system (innate and adaptive immune responses). The immune system, in the maintenance of intestinal homeostasis is called into play for the presence of good and bad luminal bacteria, food antigens, so it is continuously stimulated [1,2].

In homeostasis (condition of balance), therefore, the microbiota performs efficiently and effectively; on the contrary, in the hypothesis of “dysbiosis”, which is a perturbation of the normal homeostatic balance, the intestine loses its natural permeability and the organism falls ill more easily, firstly going through a series of acute and temporary imbalances, such as colitis, diarrhea, constipation and digestive disorders, up to a whole series (if the dysbiotic cause should persist) of digestive disorders, constipation and digestive disorders, up to a whole series (if the dysbiotic cause should persist or become chronic) of Chronic Inflammatory Bowel Diseases (IBD or MICI), including Crohn’s disease and ulcerative colitis and necrotizing enterocolitis in premature infants [1,3].

In addition to chronic inflammatory diseases, directly caused and fed by the intestinal dysbiotic state, recent studies have shown a direct correlation between dysbiosis and other extraintestinal pathologies, including diabetes, atherosclerosis, metabolic syndrome, autoimmune and neurodegenerative diseases, cardiac and circulatory disorders, atopic dermatitis, psoriasis, asthma and food allergies and intolerances [1,4-19]. But also other conditions, at first unlikely hypothesis, are actually directly correlated with intestinal dysbiosis, to the point of being concases or even elements favoring or aggravating the pathology itself: we are talking about autism [20-31], epilepsy [32-36], sleep disorders [37-38], neurodegenerative diseases [39-43], eating disorders [44-45] and obesity [46],

psychotic disorders [47-52], bipolarity [53-55] and personality disorders [56-64]. The correlation between gut dysbiosis and severe forms of Covid-19 has also recently been demonstrated [65-68].

The target of clinical treatment must therefore be the “intestinal dysbiosis” [1,69,70], in order to promote a new homeostasis (eubiosis). In the clinic, four forms of dysbiosis are recognized, each of them with a precise etiopathological and symptomatic mechanism, caused in any case by a reduction in the diversity of bacterial species, reduction of beneficial species and/or proliferation (increase) of harmful species [1]:

“putrefactive”, which originate from an increase in the share of *Bacteroides* at the expense of *Bifidobacteria*, is caused by an excessive intake of meat and saturated fats associated with a poor introduction of insoluble vegetable fibers.

“fermentative”, which originate from a poor acid secretion by the stomach associated with an overproduction of bacteria and yeasts in the stomach and small intestine, often motivated by an intolerance to gluten and carbohydrates.

“deficiency” and “sensitization”, often difficult to differentiate between them. Both forms are caused and maintained by excessive intake of toxic pollutants, antibiotic therapies and more generally by conditions that cause a decrease in the quotas of probiotic bacteria and an alteration of intestinal motility.

Dietary and integrative treatment for the management of intestinal dysbiosis: Proposed protocol (Perrotta Protocol for the clinical management of intestinal dysbiosis, PGD)

In the literature, dozens of studies have focused on the search for one or more elements that can help the intestine to regain its natural eubiosis, without ever taking into account the holistic and omnidimensional approach related to both nutritional intake and individual nutrients; in essence, the studies are always focused on the introduction of the element deficient or the element that can improve the intestinal eubiosis without ever focusing on the overall picture and the rules of management of the patient as a whole.

Thus, what follows is the result of the literature search to meet this need [71-102]. Specifically, the proposed protocol consists of four different steps:

- a) **Complete patient history:** Prepare a specific personal and family history to identify acute and chronic causes and symptomatology, and then proceed to a targeted and personalized treatment plan, using the specific questionnaire on the protocol model under review (PID-Q).
- b) **Arrangements for serum and instrumental check-up:** The patient is invited to undergo the following analyses before starting the new dietary and integrative regimen:
 - a) Baseline serum tests: CBC with leukocyte formula, erythrocyte sedimentation rate, thrombin, prothrombin, homocysteine, blood glucose, blood glucose curve,

amylase, lipase, gamma glutamyltranspeptidase, cholesterol, triglycerides, bilirubin, creatinine, uric acid, nitrogen, thyrotropin, protein electrophoresis, total immunoglobulins A-E-G-M, sodium, potassium, magnesium, iron, ferritin, vitamin b6, vitamin b12, vitamin d3.

- b) *Specific serum analyses*: to be evaluated based on patient history.
- c) *Urinary analyses*: urinary chemistry-physical, e-GFR (24h).
- d) *Complete echo-abdomen*;
- e) *Identification sequencing of intestinal microbial communities*.

Preparation of a personalized and individualized food plan that takes into account the specific allergies/intolerances of the patient. This plan [71-74] includes

- a) a balanced caloric regimen, according to the weight and age of the patient, rich in fiber (20-25 gr/dié), cereals (50 gr/dié), fruit (200-300 gr/dié), vegetable oils (e.g., extra virgin olive oil) and vegetables (600-1000 gr/dié), with abstention from the intake of sugars (simple) and/or carbohydrates (complex sugars) after 5 pm;
- b) water intake between 2000 ml / dié and 2500 ml / dié, of which at least 75% of natural mineral water;
- c) absolute abstention from the intake of the following foods packaged products (sweet and salty) and sausages of animal origin (ham, mortadella, speck, ...), products stored in aluminum or plastic cans, gluten-based or contaminated products (bread, pasta and flours), yeast (you can replace it with bicarbonate for natural leavening), sugar, salt, butter and products with a high lipid value, products cooked on the grill or burning the surface, narcotics, cigarettes and cigars;
- d) mild abstention (maximum 3 times a week) from the intake of the following foods: meat of animal origin (of which 2 times white meat and 1 time red meat), potatoes, tomatoes, eggplant, peppers, zucchini, cow's milk and derivatives (cheese made from cow's milk), products with moderate lipid value, alcohol (wine and beer, moderate amounts);
- e) moderate abstention (maximum 1 time per week) from the intake of the following foods: hard liquor (limited quantities) and products cooked in fried mode,
- f) brisk walking for 40-60 minutes, for a total of about 5000 steps/day, 3 times a week, every other day.

Administration of the supplemental regimen

A basic kit is prepared to be taken daily (unless otherwise specified) and composed of the following elements:

- ✓ Prebiotic elements (e.g., inulin and fructooligosaccharides) [77,79] and Probiotics [74,75,77,79,94,95], based on individual history, symptom severity, and type of dysbiosis encountered, administered in the indicative amount of 10-30 billion ferments/day. Particular attention to Bifidobacteria [76] and Lactobacilli [96-98] (including Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum, Bacillus subtilis, Streptococcus thermophilus, Enterococcus faecium, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus rhamnosus).
- ✓ Omega 3 (alpha-linoleic acid), 6 (oleic acid), and 9 (linoleic acid), administered in the amount of 1000-2000 mg/dié [78].
- ✓ Vitamin A (Retinol), administered in the amount of 1 mg/dié [80-82, 89]
- ✓ Vitamin B1 (Thiamine), administered in the amount of 1 mg/dié [83]
- ✓ Vitamin B2 (Riboflavin), administered in the amount of 0.5 mg/dié per 1000 Kcal introduced in the diet [83]
- ✓ Vitamin B3 (Niacin or PP), administered in the amount of 15 mg/dié [83]
- ✓ Vitamin B5 (Pantothenic Acid), administered in the amount of 5 mg/dié [83]
- ✓ Vitamin B6 (Pyridoxine), administered in the amount of 1 mg/dié [83]
- ✓ Vitamin B7 (Inositol), administered in the amount of 500 mg/dié [83]
- ✓ Vitamin B8 (Biotin), administered in the amount of 100 mg/dié [83].
- ✓ Vitamin B9 (Folic acid), administered in the amount of 2 mg/dié [84].
- ✓ Vitamin B12 (Cobalamin), administered in the amount of 1000 mg/dié [85].
- ✓ Vitamin C, administered in the amount of 1000-3000 mg/dié [86,87].
- ✓ Vitamin D3, administered in the amount of 1000-4000 IU/dié (5 days/week) + Vitamin K, administered in the amount of 1000-2000 mcg/dié (the suggested dose should be between 500 and 1000 mcg but the antagonistic efficacy of Coenzyme q10 in the protocol must be taken into account). Vitamin D3 and vitamin K may not be necessary if serum indicates this [81,85,88,89].
- ✓ Vitamin E, administered in the amount of 1000-2000 mg/dié [90]
- ✓ Curcudine, administered in the amount of 200-500 mg/dié + Piperine, administered in the amount of 5 mg or Bromelain, administered in the amount of 200 mg/dié [91]



- ✓ Quercetin, administered in the amount of 200-500 mg/dié [91]
- ✓ Lactoferrin, administered in the amount of 300-500 mg/dié [92]
- ✓ Coenzyme q10, administered in the amount of 100 mg/dié [93]
- ✓ Sodium Butyrate, administered at a dose of 1000-2000 mg/dié [103-105]
- ✓ Glutathione, administered at a dose of 500-1000 mg/dié [106].

These doses are evaluated according to a hypothetical proposal profile, based on analytical and empirical evidence from previous studies, which indicate certain thresholds of administration depending on the objectives to be achieved. Clearly, the protocol is here a proposal and therefore during future experiments it may be subject to change Table 1.

Perrotta intestinal dysbiosis clinical management protocol – Questionnaire, PID-Q

Associated with the proposed protocol, this research work also uses a specific questionnaire: Perrotta Intestinal Dysbiosis Clinical Management Protocol – Questionnaire, PID-Q.

This questionnaire is specifically structured in 4 parts in

order to have all the information needed to monitor the sample population holistically:

- 1) Section A contains the personal and contact information of each individual patient.
- 2) Section B contains the medical history of each individual patient, with particular attention to the following profiles: medical conditions during gestation, childhood and throughout development, with distinction between physical and psychological symptoms; previous and current drug therapy; specific allergies and any therapy used.
- 3) Section C contains anamnestic family data, of relatives and relatives-in-law of first and second degree, in such a way as to be able to reconstruct any family or hereditary conditions.
- 4) Section D represents the diary of the protocol, with the list of specific activities in the phase preceding the beginning of the protocol until the concluding phases, subdivided by therapies, diet and reference notes, weekly and then monthly, until the duration of the entire semester.

Appendix no. 1: Perrotta, Intestinal Dysbiosis Clinical Management Protocol – Questionnaire, PID-Q).

Table 1: Perrotta, Protocol for Clinical Management of Gut Dysbiosis - Questionnaire, PID-Q).

Specific action	Time	Indications
Complete personal and family history using the PGD-Q model	First session	<ul style="list-style-type: none"> ✓ Questionnaire completion and evaluation of past documentation
Preparation of additional serum and instrumental analyses	First session	<ul style="list-style-type: none"> ✓ basic serum tests: CBC with leukocyte formula, erythrocyte sedimentation rate, thrombin, prothrombin, homocysteine, blood glucose, blood glucose curve, amylase, lipase, gamma cholesterol, triglycerides, glutamyltranspeptidase, bilirubin, creatinine, uric acid, nitrogen, thyrotropin, protein electrophoresis, sodium, potassium, magnesium, iron, ferritin, vitamin b6, vitamin b12, vitamin d3, total immunoglobulins A-E-G-M. ✓ Specific serum tests: to be evaluated according to the patient's history. ✓ Urinary analyses: urinary chemistry, e-GFR (24h). ✓ complete echo-abdomen; ✓ Identification sequencing of intestinal microbial communities.
Receive integrative analysis	Second session	Documentary outcomes
Preparation of the personalized and individualized food plan	Third session	<p>The food plan must take into account the following specifications:</p> <ul style="list-style-type: none"> ✓ coherence with the allergic profile of the patient ✓ balanced caloric regimen, according to the weight and age of the patient, rich in fiber (20-25 g/d), cereals (50 g/d), fruit (200-300 g/d), vegetable oils (e.g. extra virgin olive oil) and vegetables (600-1000 g/d), with abstention from the intake of sugars (simple) and/or carbohydrates (complex sugars) after 5 pm; ✓ Water intake between 2000 ml/drink and 2500 ml/drink, of which at least 75% should be natural oligomineral water; ✓ Absolute abstention from the following foods: packaged products (sweet and salty) and sausages of animal origin (ham, salami, mortadella, speck, ...), products preserved in aluminum or plastic cans, gluten-based or contaminated products (bread, pasta and flours), yeast (you can replace it with bicarbonate for the natural leavening), sugar, salt, butter and high-fat products, products cooked on the grill, drugs, cigarettes and cigars; ✓ Slight abstention (maximum 3 times per week) from the intake of the following foods: meat of animal origin (of which 2 times white meat and 1 time red meat), potatoes, tomatoes, eggplants, peppers, zucchini, cow's milk and derivatives (cheese made from cow's milk), products with moderate lipid value, alcohol (wine and beer, moderate quantities); ✓ moderate abstention (maximum 1 time per week) from the intake of the following foods: spirits (limited quantities) and products cooked in fried mode, ✓ brisk walking for 40-60 minutes, for a total of about 5000 steps/day, 3 times a week, every other day.



Preparation of the supplementary scheme	Third session	<p>Preparation of the basic kit to be taken daily, unless otherwise indicated:</p> <ul style="list-style-type: none"> ✓ Prebiotics/Probiotics, 10-30 billion/day ✓ Omega 3 (alpha-linoleic acid), 6 (oleic acid) and 9 (linoleic acid), 1000-2000 mg/dié ✓ Vitamin A (Retinol), 1 mg/day ✓ Vitamin B1 (Thiamine), 1 mg/day ✓ Vitamin B2 (Riboflavin), 0.5 mg/dié per 1000 Kcal introduced with the diet ✓ Vitamin B3 (Niacin or PP), 15 mg/day ✓ Vitamin B5 (Pantothenic Acid), 5 mg/day ✓ Vitamin B6 (Pyridoxine), 1 mg/day ✓ Vitamin B7 (Inositol), 500 mg/day ✓ Vitamin B8 (Biotin), 100 mg/day ✓ Vitamin B9 (Folic Acid), 2 mg/day ✓ Vitamin B12 (Cobalamin), 1000 mg/day ✓ Vitamin C, 1000-3000 mg/day ✓ Vitamin D3, 1000-4000 IU/day (5 days/week) + Vitamin K, 1000-2000 mcg/day ✓ Vitamin E, 1000-2000 mg/day ✓ Curcudine, 200-500 mg/day + Piperine, 5 mg or Bromelain, 200 mg/day ✓ Quercitin, 200-500 mg/day ✓ Lactoferrin, 300-500 mg/day ✓ Coenzyme Q10, 100 mg/day ✓ Sodium Butyrate, 1000-2000 mg/dié ✓ Glutathione, 500-1000 mg/dié
Start of administration of the new dietary and supplementary regime	Fourth session	Start of administration
Weekly follow-up for the first trimester	Fifth session	Monitoring of symptomatology and tolerance to the protocol for a total period of 3 months
Administration of serum and instrumental analyses	Sixth session	Serum and instrumental evaluation, by the end of the first trimester, unless otherwise clinically indicated
Follow-up / secondo trimestre	Seventh session	Monitoring of symptoms and regularity of the protocol for an additional period of 3 months
Administration of serum and instrumental analyses	Eighth session	Serum and instrumental evaluation, by the end of the second trimester, unless otherwise clinically indicated

Concluding considerations

The application of this proposal as an experiment could bring very interesting results, in the light of scientific literature, both in the acute phase (for the resolution of symptoms or as a preventive function) and in the chronic phase (for the management of the morbid condition and its clinical consequences). Awaiting further developments, we await new research able to identify the exact distribution of all microorganisms that are part of the microbiota and the complete mapping of the microbiome.

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