Study protocol

A prospective, proof-of-concept, clinical study on gut microbiota in patients with metabolic syndrome, type 2 diabetes and obesity

Study Type:	A prospective, proof-of-concept, interventional study on gut microbiota composition and function in patients with metabolic syndrome,and/or type 2 diabetes and/or obesity treated with standard therapy
Sponsor-investigator:	Profit study
Local investigators/ subinvestigators	Principal Investigator: Prof. Antonio Gasbarrini Sub-Investigators: to be defined
Protocol Version and Date:	Version 1, 08th July 2019
Trial management and coordinating facility:	Fondazione Policlinico Universitario Agostino Gemelli IRCCS Center for Digestive Diseases Internal Medicine and Gastroenterology Unit Largo Agostino Gemelli, 8 00168 Rome Italy

CLINICAL STUDY PROTOCOL INVESTIGATOR SIGNATURE PAGE

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The Principal-Investigator has approved the protocol version dated and confirms hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines, ISO 14155 norm and the local legally applicable requirements.

Principal-Investigator:

Date _____ Signature _____

1. Background

Gut microbiota and metabolic syndrome (MS)

Recently, the role of gut microbiota in metabolic syndrome and associated diseases gained interest among researchers. Metabolic syndrome is defined by a clustering of metabolic disorders that include central adiposity with visceral fat accumulation, dyslipidemia, insulin resistance, dysglycemia and non-optimal blood pressure levels and it is associated with an increased risk of cardiovascular diseases and type 2 diabetes. In fact, gut microbiota could have a role in regulation of body weight and energy homeostasis, influencing extraction of calories from food and their storage in host adipose tissue. Furthermore, gut microbiota in obese and lean individuals differs in the ability to play these functions[1]. At the moment, the first line standard therapy for MS consists in dietary and lifestyle intervention.

2. Objectives

The primary aim of this study is to evaluate the effects on microbiota of current standard medical treatment for MS, type 2 diabetes and obesity, including microbiome composition characterization based on metagenomic analysis of stools and saliva and genotyping of blood and functional characterization based on metabolomics analysis on urine and blood.

Secondary aims will include clinical efficacy of treatments and correlation with microbiome and metabolome characterization.

3. Trial design

3.1 Overall study design

This is prospective, proof-of-concept, inteventional clinical study. The interventional part of the study is not related to treatment –that will be followed as per standard clinical practice- but to monitoring, that is based on several biologic samples, to understand the possible effect on microbiota of standard medical treatment. Three cohorts of approximately 33 consecutive patients will be included, to reach overall 100 patients included:

1. patients with type 2 diabetes without obesity

2. patients with type 2 diabetes and obesity

3. patients with obesity but without type 2 diabetes

. Furthermore, a group of 50 healthy controls, matched for sex and age with patients, will be enrolled to allow comparison in microbiota composition and function. Healthy controls will only provide samples at one single timepoint. Patients will be required to collect samples following the

attached flow chart (see below, section 4). All patients will be treated with first line standard medical treatment. Standard medical treatment includes dietary advice and lifesyle modification to increase aerobic physical activity. For type 2 diabetes, first line treatment could also include metformin.

Study duration for all patients will be 60 days + 14 days of screening phase.

3.2 Recruitment

Suitable subjects will be identified from patients referred to the Center of Digestive Diseases at the Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome Italy. Based on the number of patients that are referred to the clinic, we estimate that 4-5 patients per week will be recruited. The approximate time for recruitment is about 20 weeks. The potential study participants will receive oral and written information about the study. Patients that agree to participate in the study will be asked to sign a written informed consent.

3.3 Inclusion criteria

The participants have to fulfill the following criteria for participating in the study:

- Age 18 to 65 years
- Willingness to participate to the study
  Cohort 1: patients with type 2 diabetes without obesity

- diagnosis of diabetes according to the American Diabetes Association[4]

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h PG ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.*
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma

glucose ≥200 mg/dL (11.1 mmol/L).

* In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples.

- BMI <30 kg/m^2 or waist circumference <94 cm in men and 80 cm in women[3]

Cohort 2: patients with type 2 diabetes and obesity

- diagnosis of diabetes according to the American Diabetes Association[4]

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h PG ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.*
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

* In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples.

- BMI >30 kg/m^2 or waist circumference >94 cm in men and 80 cm in women[3]

- **Cohort 3: patients with obesity but without type 2 diabetes**
- : BMI >30 kg/m^2 or waist circumference >94 cm in men and 80 cm in women[3].

FPG or 2-h PG not meeting criteria for diagnosis of diabetes

Healthy controls

- Age 18 to 65 years
- No history of systemic or gastrointestinal diseases

- No currently smokers
- No alcohol or drug abuse history
- No history of concomitant medications
- Regular bowel habits
- No history of bowel surgery
- Normal BMI (18- 25)

3.4 Exclusion criteria

- Patients who have already started first-line standard medical treatment for obesity or type 2 diabetes
- Active gastrointestinal infections
- Chronic gastrointestinal diseases
- Pregnancy and breastfeeding
- Drug or alcohol abuse
- Bulimic or binge eating pattern
- Psychiatric or cooperative problems or low compliance that is a contraindication from participating in the study.

3.5 Sample size evaluation and statistical analysis

This is a proof-of-concept study, thus no statistical evaluation of sample size has been performed. We plan to enroll 100 consecutive patients and 50 healthy controls.

4. Summary of measurements and data collection

	Screening	Inclusion (start of treatment/diet)	Visit 1- day 30	Visit 2- day 60
Visit window	-14 to -2	0	+ 28 to +32	+ 58 to + 62
Written informed consent	X			
Patient's demographics, clinical and family history	X			
Concomitant medications (including OTC and illegal drugs)	X	X	X	X
Nutrition and dietary habits	X	X	X	X
Smoking habits	X	X	X	X
Physical activity habits	X	X	X	X
Inclusion and exclusion criteria evaluation	X	X		
Physical examination	X	X	X	X
Anthropometric measurements	X		X	X

(including weight, height and waist circumference)		X		
Stool sample for metagenomics	X	X	X	X
Saliva sample for metagenomics	X	X	X	X
Urine sample for metabolomics		X	X	X
Blood sample for metabolomic		X	X	X
Blood sample for genotyping		X	X	X
Response to treatment evaluation			X	X

5. Bibliography

1. Mazidi M, Rezaie P, Kengne AP, et al. Gut microbiome and metabolic syndrome. Diabetes & metabolic syndrome 2016;**10**(2 Suppl 1):S150-7 doi: 10.1016/j.dsx.2016.01.024[published Online First: Epub Date]|.
2. Sheehan D, Shanahan F. The Gut Microbiota in Inflammatory Bowel Disease. Gastroenterology clinics of North America 2017;**46**(1):143-54 doi: 10.1016/j.gtc.2016.09.011[published Online First: Epub Date]|.
3. WHO. Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. Geneva, 2008.
4. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. Diabetes care 2019;**42**(Suppl 1):S13-S28 doi: 10.2337/dc19-S002[published Online First: Epub Date]|.
5. Secondary 2019. https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome.