Risk of Microbial Translocation in patients undergoing Per-Oral Endoscopic Myotomy (POEM) for Achalasia: Antibiotic Prophylaxis or Short-therapy?

Promotore della sperimentazione: Dr. Roberta Maselli

Codice dello studio: POEM-MT01

Version N° 1- 15 Oct 2016

Contact addresses

Principal investigator:	Dr. Roberta Maselli Phone: 02-82247570 Fax: 02-82242292 e-mail: roberta.maselli@humanitas.it
Study Coordinator:	Dr. Roberta Maselli Phone: 02-82247570 Fax: 02-82242292 e-mail: roberta.maselli@humanitas.it
Institutional Address	IRCCS Humanitas Research Hospital Via A.Manzoni 56 20089 Rozzano, Milano
Collaborating center	Università degli Studi di Roma “La Sapienza” Piazzale Aldo Moro 5 00185 Roma

Signatures Page

STUDY COORDINATOR SIGNATURE *(where applicable)*

_____
Printed name

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Role & Department

_____ _____
Signature Date

CENTRE SIGNATURE – PRINCIPAL INVESTIGATOR

I have read this Protocol Amendment relevant to the study entitled *"Risk of Microbial Translocation in patients undergoing Per-Oral Endoscopic Myotomy (POEM) for Achalasia: Antibiotic Prophylaxis or Short-therapy?"* and I agree to conduct the study as detailed herein and in compliance with guidelines for Good Clinical Practice and applicable regulatory requirements. I will provide all study personnel under my supervision with all information provided by the Sponsor and I will inform them about their responsibilities and obligations.

_____
Printed name

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Role & Department

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Address

_____ _____
Signature Date

INDEX

Summary

Title	Risk of Microbial Translocation in patients undergoing Per-Oral Endoscopic Myotomy (POEM) for Achalasia: Antibiotic Prophylaxis or Short-therapy
Study coordinator	Dr. Roberta Maselli
Protocol identifying code	POEM-MT01
Protocol version date	Version N° 1. 15 Oct 2016
Background and rationale	Achalasia is a primary rare esophageal motor disorder of the esophagus (annual incidence of 1:100,000 persons). Recently, a new endoscopic technique, Per-Oral Endoscopic Myotomy (POEM), has been introduced with excellent success rates. Several studies have evaluated complications of POEM but there is a lack of knowledge on the potential risk of bacteremia or microbial translocation during the endoscopic intervention and, also, there aren't evidences regarding the use of antibiotics before/after POEM. Microbial translocation (MT) is the passage of both viable and nonviable microbes across the anatomically intact GI barrier to the mesenteric lymph nodes, and possibly other tissues. Gram-negative bacteria contain lipopolysaccharides (LPSs) coating their thin peptidoglycan cell wall. The presence of LPS, an endotoxin, in the plasma has been correlated to sepsis and septic shock through the activation of the inflammatory host defence via binding to soluble CD14 (sCD14) which initiates downstream cytokines (like IL-6, IL-8 and tumor necrosis factor (TNF-α)) and, also, through the production of sCD14 and LPS-binding protein (LBP) by the innate immune system. Considering POEM a clean-contaminated procedure, it should be assessed whether the post-POEM fever or systemic inflammation is a cytokinin-mediated or an infection-related fever. Thus, aim of the study is to evaluate the presence of inflammation mediators, bacteremia and microbial translocation post POEM, to guide future antibiotic prophylaxis/therapy in patients undergoing this procedure.
Population and patient selection criteria	Study population: patients with esophageal achalasia (diagnosed accordingly to the international guidelines) scheduled for POEM, who are willing to partecipate in a RCT and who are able to give informed consent. Inclusion citeria: 1. Patients who had a diagnosis of achalasia defined by symptoms, HR manometry and X-ray accordingly to guidelines, who have no absolute contraindications to undergo interventions under general anesthesia 2. Age over 18 years 3. Ability to provide and to give informed consent 4. Body temperature under 37 °C on the day before and just before POEM Exclusion criteria: 1. Antibiotic use within 1 week before the procedure

	2. Patients who had chronic inflammatory diseases (such as rheumatic arthritis or inflammatory bowel diseases) and/or known neoplasia 3. Inability to obtain written informed consent 4. Patient unwilling to participate to the study 5. Impossibility to be subjected to the invasive endoscopic procedure or general anesthesia for the presence of comorbidities
Study design and study duration	In this study we will enroll all consecutive patients with esophageal achalasia, diagnosed by clinical symptoms, esophageal HR manometry and X-ray barium swallow (accordingly to the international guidelines) evaluated during a gastroenterological outpatient visit. These patients will undergo POEM at our institution from June 2017 to June 2019. Duration of the study (patient enrolment; treatment duration, follow up, etc.): we expected six months for the approval of the study by the Ethics Committee of our Institute, personnel training, study protocol registration; twenty-four months of enrollment, interventions and follow-up; six months for processing data and for the drafting of the final manuscript.
Description of study treatment/product/intervention	All patients who will undergo POEM at Humanitas Research Hospital from June 2017 to June 2019 will be enrolled in a prospective, interventional randomized clinical trial (RCT). Patients will be randomized in two groups. The Group A, prophylaxis group, will receive antibiotics (Cefazoline 2 gr i.v.) only before procedure whereas Group B, short therapy group, will receive antibiotics before POEM (Cefazoline 2 gr i.v.), continued for the first 24 hours and then per os (Amoxicilline/Clavulanic Acid 3 gr/die) for 3 days. For each patient we will be evaluated: plasma dosage of IL-6, IL-1β, TNF-α, sCD4, LPB, LPS and blood cultures.
Objectives	Considering POEM a clean-contaminated procedure, it should be assessed whether the post-POEM fever or systemic inflammation is a cytokinin-mediated or an infection-related fever. Thus, aim of the study is to evaluate the presence of inflammation mediators, bacteremia and microbial translocation post POEM, to guide future antibiotic prophylaxis/therapy in patients undergoing this procedure.
Statistical methods, data analysis	From a retrospective internal analysis on POEMs performed from October 2015 to October 2016 in our istitution we estimated that, in patients submitted to short antibiotic therapy, the prevalence of fever and/or systemic inflammation is about 30%. We want to demonstrate that giving only prophylaxis antibiotics, the prevalence of fever and/or systemic inflammation will be not higher than 10% difference with and alpha error of 5% and a power of 80%: we need 62 patients each group, for a total of 124 patients. Data will be described as number, percentage and confidence interval, or mean, standard deviation and confidence interval, or

	median and interquartile interval where appropriated. Differences between groups will be explored with chi square test, with fisher correction if appropriated with qualitative data, or with wilcoxon test or t student test if quantitative. No interim analysis is planned.
Study time table	- duration of the study (patient enrolment; treatment duration, follow up, etc.): we expected six months for the approval of the study by the Ethics Committee of our Institute, personnel training, study protocol registration; twenty-four months of enrollment, interventions and follow-up; six months for processing data and for the drafting of the final manuscript. - when the final report will be provided: December 2019 - overall study duration from the First Patient First Visit (FPFV): twenty-four months plus five days

1 Background and introduction

Achalasia is a primary rare esophageal motor disorder of the esophagus with an estimated annual incidence of 1:100,000. It is characterized by the absence of esophageal peristalsis and by failure of the lower esophageal sphincter to relax upon swallowing. Recently, a new endoscopic technique, POEM has been introduced as an alternative treatment to surgery with excellent success rates.

Several studies have evaluated complications of POEM such as bleeding, perioperative perforation, pneumothorax, pneumoperitoneum, but there is a lack of knowledge on the potential risk of bacteremia or microbial translocation (MT) during the endoscopic intervention.

Moreover, several reviews reported the routine use of pre-procedure antibiotic prophylaxis for POEM with no specifying regarding the type and dosage of antibiotic . The latest ASGE (American Society od Gastrointestinal Endoscopy) guidelines on antibiotic prophylaxis for GI (gastro-intestinal) endoscopy do not illustrate the management of antibiotic for POEM. On the contrary, in clinical practice most centers use to administer antibiotics just after the procedure; no evidences have been published regarding the use of antibiotics before/after POEM.

The reason for the development of post-POEM fever or systemic inflammation has not been investigated yet. It is possible that the long procedure time and contact time of electric knives to the submucosal layer could potentially promote GI bacteria translocation. Moreover, the extensive submucosal defect caused by POEM exposes deep GI layers to the indigenous bacterial flora which may theoretically cause microbial translocation and/or bacteremia.

The presence of bacteremia during gastrointestinal endoscopy has been established in several studies. MT is the passage of both viable and nonviable microbes across through the anatomically intact GI barrier to the mesenteric lymph nodes, and possibly other tissues. All bacteria including those colonizing the gut can be divided into two groups, Gram-negative and Gram-positive bacteria, depending on their cell wall structure and outer membrane. Gram-negative bacteria contain LPSs coating their thin peptidoglycan cell wall. Over the past decades, the presence of LPS, commonly referred to as endotoxin, in the plasma has been correlated to multiple diseases including sepsis and septic shock. LPSs presence in the peripheral circulatory system could thus signify potential bacterial invasion, requiring rapid host response. Potent pyrogens, endotoxins activate the inflammatory host defence via binding to sCD14 (sCD14), which initiates downstream cytokines important for the clearance of bacterial infections.

Additionally to the circulating IgM, IgA, and IgG antibodies directed against the LPS core antigen that neutralize its activity, the innate immune system produces soluble factors, such as sCD14, and LBP. CD14 is an LPS coreceptor expressed by peripheral blood monocytes and tissue macrophages. Following LPS stimulation, CD14+ monocytes/macrophages secrete sCD14 and shed surface CD14, which binds to LPS.

Taken together, it is clear that several circulating factors, including sCD14 and LBP act as fundamental lines of defence against systemic stimulation of the immune system by translocated microbial antigens.

When CD14 transfers LPS to monocytes two specific signalling pathways are activated both leading to the recruitment of numerous downstream molecules, which finally results in the transcription of proinflammatory cytokines such as interleukin IL-6, IL-1β and TNF-α.

Since the manifestation of febrile state is dependent upon the pro-inflammatory response towards endotoxins, sCD14, LPS and LBP could be used as a suitable biomarker associated with microbial translocation, and IL-6, IL-1β and TNF-α as a biomarkers of inflammatory pattern.

2 Rationale of the study

Literature does not describe a unique management toward antibiotic prophylaxis of patients that must undergo POEM. The same guidelines on antibiotic prophylaxis for GI endoscopy do not illustrate the management of antibiotic for POEM. Several reviews reported the routine use of pre-procedure antibiotic prophylaxis for POEM (without specifying the type of antibiotic and dosage) but, on the contrary, in clinical practice most centers use to administer antibiotics just after the procedure.
Our study, by evaluating the presence of inflammation mediators, bacteremia and microbial translocation post POEM, would like to investigate the origin of the post-POEM fever or post-POEM systemic inflammation (if these are cytokinin-mediated or infection-related).

In this way we could:
- standardize the management of antibiotic therapy for patients undergoing POEM
- allow adequate treatment selection and avoid unnecessary antibiotic usage
- reduce the risk of antibiotic resistance
- optimize the length of hospitalization

3 Objectives of the study

The primary objective of our study is determine whether fever (TC > 38°C) and systemic inflammation (increased in white blood cells (WBC) and/or protein C-reactive (PCR)) that may occur following POEM, are cytokine-mediated or infection-related and whether they are affected by prophylaxis or antibiotic short therapy.

The secondary objectives of our study are:

- assess the presence of post-POEM bacteremia though the result of blood cultures

- assess the presence and quantify the degree of post-POEM microbial translocation through the dosage sCD4, LPB and LPS

- quantify the degree of post-POEM systemic inflammation through the dosage of IL-6, IL-1β and TNF-α

Statistical hypotesis: the aim of the study is to evaluate the presence of inflammation mediators, bacteremia and microbial translocation post-POEM, to guide future antibiotic prophylaxis/therapy in patients submitted to POEM. The study wants to assess the non-inferiority of antibiotics prophylaxis versus antibiotics short therapy in terms of fever and/or systemic inflammation control.

4 Patient selection criteria

Study population: patients older than 18, with esophageal achalasia (diagnosed accordingly to the international guidelines) scheduled for POEM, who are willing to partecipate in a RCT and who are able able to give informed consent.

Clinical setting: the study will be conducted at the institute IRCCS Humanitas Research Hospital (Rozzano, Milano) at the operating unit of Gastroenterology and Digestive Endoscopy and at the Institute Sapienza University of Rome, Policlinico Umberto I° at the Department of Public Health and Infectious Diseases.

Enrollment procedure: the investigator or his/her representative who will perform the procedure will explain the nature of the study to the subject and answer all questions regarding the study, in

particular he/she will illustrate the purpose of the study, the absence of risk for the patient, making it clear that the current procedure of POEM will not be changed. The subjects review, signed and dated the informed consent (a copy of the IC form will be given to the subject and the original placed in subject medical form).

Accrual time: from June 2017 to June 2019 (patients enrollment)

In our study we will not be involved vulnerable populations.

4.1 Inclusion criteria

1. Patients who had a diagnosis of achalasia defined by symptoms, HR manometry and X-ray accordingly to guidelines, who have no absolute contraindications to undergo interventions under general anesthesia
2. Age over 18 years
3. Ability to provide and to give informed consent
4. Body temperature under 37 °C on the day before and just before POEM

4.2 Exclusion criteria

1. Antibiotic use within 1 week before the procedure
2. Patients who had chronic inflammatory diseases (such as rheumatic arthritis or inflammatory bowel diseases) and/or known neoplasia
3. Inability to obtain written informed consent
4. Patient unwilling to participate to the study
5. Impossibility to be subjected to the invasive endoscopic procedure or general anesthesia for the presence of comorbidities

5 Study Design

In this study we will enroll all consecutive patients with esophageal achalasia, diagnosed by clinical symptoms, esophageal HR manometry and X-ray barium swallow (accordingly to the international guidelines) evaluated during a gastroenterological outpatient visit. These patients will undergo POEM at our institution from June 2017 to June 2019.

In our center, these patients usually undertake a preoperative routine (blood tests, cardiological evaluation, chest x-rays and anesthesia assessment) some weeks earlier, in order to test the feasibility of general anesthesia for the endoscopic invasive procedure. Patients are admitted to the gastroenterology department the morning of the procedure and, therefore, they are accompanied in the operating room.

The POEM procedure will be carried out according to the current clinical practice. After an EGDS a solution is endoscopically injected in the mid esophagus to produce a large cushion, thereby promoting a safe mucosal incision. After submucosal injection a longitudinal large incision is performed to allow the entrance of the endoscope into the submucosal layer. Then, the submucosal layer is longitudinally dissected to create a tunnel from the esophageal submucosal incision to the gastric side. The created tunnel will be the working space to perform the myotomy. During the tunnelling any possible bleeding is promptly endoscopically treated. The endoscopic myotomy (ether selective of the circular muscle layer or both circular and longitudinal muscle layer) is then performed. Finally the mucosal incision is clipped by standard endoscopic clips.

After the procedure, patients are moved to the ward where they received the appropriate supportive care (pain reliever and antiemetic as needed, intravenous hydration and IPP) and they remain

fasting. The next day blood tests are performed to rule out the occurrence of major complications; esophageal X-Ray are performed where needed. Then patients resume to eat with liquid diet and then with a soft diet. Finally, on the same day, in the absence of complications patients is discharged. Our current routine provides antibiotic coverage with Cefazolin ev during the entire duration of stay.
When performing the clinical laboratoristic work-up, subjects will be evaluated on the basis of the inclusion and exclusion criteria. They will be proposed for the study and, if they agree to participate, they will sign the informed consent after adequate explanations. All patients who agree to participate in the study will be randomized, by a 1:1 randomization when arrived in the operating room (T0). At the same time, first blood samples will be collected for plasma cytokine dosage (IL-6, IL-1β, TNF-α, sCD4, LPB and LPS) and bacteremia. Patients randomized to the group A, prophylaxis group, will receive a dose of antibiotics (Cefazoline 2 gr iv) 30 minutes before the procedure and patients randomized to the group B, short therapy group, will receive a dose of antibiotics (Cefazoline 2 gr iv); in this latter group the antibiotic will be also continued during hospitalization and at home (Cefazoline 6gr/day for the first 24 hours then orally Amoxicilline/Clavulanic Acid 3 gr/day for 3 days). For patients allergic to beta-lactams, a different class of antimicrobials will be prescribed according to the Institution's clinical practice and patient's characteristics.
At the end of the procedure (T1) and at 24 hours after procedure (T2) the second and the third blood samples, respectively, will be carried out (for bacteremia, IL-6, IL-1β, TNF-α, sCD4, LPB and LPS). In addition, blood samples will be performed to evaluate white blood count and PCR, as routinely done 24 hours after POEM. Additional blood cultures will be performed in case of fever (body temperature> 38 °C). It is finally scheduled a phone call five days after the procedure to complete the clinical follow-up and to ensure the compliance of recruitment of therapy for patients enrolled in group B.

6 Study treatment

Patients randomized to group A (prophylaxis group) will undergo a prophylactic antibiotic therapy with Cefazoline 2 gr administered by intravenously, 30 minutes before the procedure. Instead, patients randomized in the group B (short therapy group) will be subjected to a broader antibiotic coverage characterized by Cefazoline 2 gr i.v. 30 minutes before the procedure and then continued for the next 24 hours (Cefazoline 6 gr/ day i.v.) and, thereafter, orally for the next 3 days (Amoxicilline / Clavulanic Acid 3 gr / day). The dosage of antibiotics will be modified according to creatinine clearance. For patients allergic to beta-lactams, a different class of antimicrobials will be prescribed according to the Institution's clinical practice and patient's characteristics.
In case the POEM will last for more than 4 hours, an additional administration of Cefazoline (2 gr i.v.) will be given.

The object of our study is not to investigate the efficacy and safety of a new drugs. The drugs that we will use (Cefazoline, Amoxicilline/Clavulanic Acid) are commonly used in clinical practice and in the management of patients who undergo POEM, and part of GCP (Good Clinical Practice).

Patient compliance will be monitored by means of a telephone survey.

Stopping rules/discontinuation criteria: in our study will be not used experimental drugs, the patient will leave the study only in case of occurrence of allergic reaction to the drug used.

7 Clinical evaluation, laboratory tests and follow-up

For each subject, blood samples will be collected at T0, T1, T2 for the following analyses:
- 5 ml of whole blood will be inoculated on blood cultures bottles and will be transferred to the Humanitas Microbiology Laboratory within 3h from collection and processed as daily practice to evaluate the presence of bacteria in the blood;
- 5 ml of whole blood sample for plasma collection. Briefly, plasma will be collected from EDTA whole blood samples within 6 hours of collection by centrifugal force of 1000g for 20 mins, will be then removed and stored at -80°C until the enzyme-linked immunosorbent assays (ELISA) for IL-6, IL-1β, TNF-α, sCD4, LPB and LPS will be performed. Experiments will be performed in duplicate.

In the event that the patients develop fever accompanied by clinical signs of sepsis or develop a complication to the procedure (eg, aspiration pneumonia) specific changes of antibiotic therapy will be administered according to the clinical judgment of the physician. In case of positive blood culture to a specific bacterium, a specific therapy will, therefore, guided by antibiogram.

There aren't drugs not allowed.

8 Statistical considerations

Randomization: patients will be randomized by a 1:1 randomization.
Blinding: not applicable.

From a retrospective internal analysis on POEMs performed from October 2015 to October 2016 in our institution we estimated that, in patients submitted to shot antibiotic therapy, the prevalence of fever and/or systemic inflammation is about 30%. We want to demonstrate that giving only prophylaxis antibiotics, the prevalence of fever and/or systemic inflammation will be not higher than 10% difference with and alpha error of 5% and a power of 80%: we need 62 patients each group, for a total of 124 patients.
Data will be described as number, percentage and confidence interval, or mean, standard deviation and confidence interval, or median and interquartile interval where appropriated. Differences between groups will be explored with chi square test, with fisher correction if appropriated with qualitative data, or with wilcoxon test or t student test if quantitative.
No interim analysis is planned.

9 Forms and procedures for collecting data and data managing

Data that will be gathered: indicators of systemic inflammation and of microbial translocation, presence of bacteremia, procedure's complications.
The forms/tools used for the retrieval of information and their validity and reliability: all the tools used to obtain the data have been validated and certified annually by clinical engineering of our institute and the calibration, adequacy and validation certificates are preserved by them and can be consulted in case of need.
The measures/indicators used: plasma dosage of sCD14, LPS, LBP, IL-6, IL-1β and TNF-α, results of blood cultures, presence of fever (TC > 38°C), increase in inflammatory markers (white blood cells and PCR).
The potential sources of biases in the retrieval of information regarding study subjects and interventions/treatments: any possible missing data, because for example some of the participants could drop out before the end of the study, because participants could not follow the protocol either

deliberately or accidentally, or because some outcomes could be not measured correctly or cannot be measured at all at one or more time points.
Duration and frequency of follow up: follow-up is performed with a phone call five days after the procedure.
Expected estimate of subjects lost to follow up and possible implications for the findings of the study: 5% estimated lost to follow up; this will not affect the result of the study because the aim of the study is mainly focalized on the first 24 hours after POEM, when the patients is still hospitalized.
Potential confounding factors and methods for taking into account their effect: with possible underlying co-infections could confound the results of our study, for this reason will not be enrolled patients with fever or patient who had taken antibiotics until 1 week before the procedures. Other potential confounders: patients with biochemical signs of inflammations/infections (increased PCR and/or WBC count), for this reason patients with any known systemic chronic inflammation disease or neoplasia will not be enrolled.
Identification of source data definition (electronic clinical reporting form (e-CRF)): N/A because we will not use e-CRF but a paper CRF for records. Data will be finally collected in a specific electronic database and validated by an external monitor.

10 Ethical considerations

10.1 Patient protection

The responsible investigator will ensure that this study is conducted in agreement with either the Declaration of Helsinki (Tokyo, Venice, Hong Kong and Somerset West amendments) or the laws and regulations of the country, whichever provides the greatest protection of the patient.

The protocol has been written, and the study will be conducted according to the ICH Guideline for Good Clinical Practice

The protocol and its annexes are subject to review and approval by the competent Independent Ethics Committee(s) (“IEC”).

10.2 Subject identification – Personal Data protection

All records identifying the subject must be kept confidential and, to the extent permitted by the applicable laws and/or regulations, not be made publicly available. The name of the patient will not be asked for nor recorded at the Data Center. A sequential identification number will be automatically attributed to each patient registered in the study. This number will identify the patient and must be included on all case report forms. In order to avoid identification errors, patient initials and date of birth will also be reported on the case report forms.

Any and all patient information or documentation pertaining to a clinical trial, to the extent permitting, through a “key” kept anywhere, regardless of whether such key is supplied along with the information or documentation or not, must be considered as containing sensitive personal data of the patient, and is therefore subjected to the provisions of applicable data protection (“privacy”) regulations. Breach of such regulations may result in administrative or even criminal sanctions.

Particularly, an information sheet prepared according to such regulations and a form to evidence the consent of patients to the processing of such data must therefore accompany the informed consent administered to the patient (see paragraph 14.3 below). Such information must (i) identify the roles of the holder (“titolare”) and processor (“responsabile”, appointed by the holder) of the patient personal data (also if not directly identifying the patient), as well as the purposes of the personal

data collection and processing (medical treatment and related/unrelated scientific research), (ii) adequately describe the flows of communication involving them, particularly if third parties should become involved, and (iii) seek the patient's prior and specific consent to such processing.

Patient information or documentation may be considered "anonymous", and as such not subject to privacy regulations, only when no key whatsoever, permitting the identification of the patient, is any longer available.

Particular attention should therefore be paid (and information/consent materials adapted accordingly) whenever patient data are supplied to third parties and may be autonomously processed, or biological samples/materials are taken and kept for future research purposes, associated or not with the pathology considered in the study.

10.3 Informed consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for study purposes by authorized individuals other than their treating physician. An example of a patient informed consent statement is given as an appendix to this protocol.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered or randomized at the Data Center. This must be done in accordance with the national and local regulatory requirements.

For European Union member states, the informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

A copy of Informed consent should be attached to this Protocol Template.

11 Conflict of Interest

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest.

12 Data ownership

According to the ICH Guidelines on Good Clinical Practice the sponsor of a study (the Institution, should the investigator or study coordinator act as sponsor in the performance of her/his institutional duties under the employment or collaboration agreement with Humanitas) is the owner of the data resulting therefrom. All centers and investigators participating in the study should be made aware of such circumstance and invited not to disseminate information or data without the Institution's prior express consent.

13 Publication Policy

After completion of the study, the project coordinator will prepare a draft manuscript containing final results of the study on the basis of the statistical analysis. The manuscript will be derived to the co-authors for comments and after revision will be sent to a major scientific journal.

All publications, abstracts, presentations, manuscripts and slides including data from the present study will be submitted to and reviewed by the Study Coordinator for coordination and homogeneity purposes: specific advance periods for submission and review may be specified in the protocol. The timing of publications (in the event several Centers should be participating in the Study) may be coordinated, and publication delayed if patentable inventions should be involved (for the time required in order to file the relevant patent applications); otherwise, according to the MoH's Decree of May 12, 2006, investigators cannot be precluded from or limited in publishing the results of their studies (IECs must verify that no excessive restriction is contained in the protocols submitted to their review and approval).

14 Study time table

Duration of the study (patient enrolment; treatment duration, follow up, etc.): we expected six months for the approval of the study by the Ethics Committee of our Institute, personnel training, study protocol registration; twenty-four months of enrollment, interventions and follow-up; six months for processing data and for the drafting of the final manuscript.

Final report will be provided: December 2019

Overall study duration from the First Patient First Visit (FPFV): twenty-four months plus five days

The study is designed in accordance with GCP and for this reason we do not foresee any additional risks for the patients rather than known antibiotics- and procedures-related risks.

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