A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL STUDY OF THE EFFICACY OF INTRAVENOUS CLARITHROMYCIN AS ADJUNCTIVE TREATMENT IN PATIENTS WITH SEPSIS AND RESPIRATORY AND MULTIPLE ORGAN DYSFUNCTION SYNDROME

Running title: INtravenous CLArithromycin in Sepsis and Multiple Organ Dysfunction Syndrome (INCLASS study)

CLINICAL STUDY PROTOCOL

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DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED CLINICAL STUDY OF THE EFFICACY OF INTRAVENOUS CLARITHROMYCIN AS ADJUNCTIVE TREATMENT IN PATIENTS WITH SEPSIS AND RESPIRATORY AND MULTIPLE ORGAN DYSFUNCTION SYNDROME.

The herein protocol became known to myself by the Study Sponsor. I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.

The Principal Investigator,

Print Name

Signature Date

ABBREVIATIONS

(S)AE: (serious) adverse event

APACHE: acute physiology and chronic health evaluation

ARDS: acute respiratory distress syndrome

BSI: primary Gram-negative bacteremia

CCI: Charlson comorbidity index

CD4: T4 lymphocytes

COPD: chronic obstructive pulmonary disease

CRF: case report form

DRGs: diagnosis related groups

EDTA: ethylenediamine actic acid

EGFR: epithelial growth factor receptor

EIA: enzyme immunoassay

G: grammar

HAP: hospital-acquired pneumonia

HCAP: health care-associated pneumonia

HIV: human immunodeficiency virus

HPLC: high-performance liquid chromatography

IAI: intra-abdominal infection

ICER: incremental cost-effectiveness ratio

ICF: informed consent form

ICU: intensive care unit

IL: interleukin

MODS: multiple organ dysfunction syndrome

OR: odds ratio

PaO_2/ FiO_2: partial arterial oxygen pressure per fraction of inspired oxygen

PBMCs: peripheral blood mononuclear cells

QALY: quality-adjusted life-year

RCT: randomized clinical trial

SOFA: sequential organ failure assessment

SUSAR: suspected, unexpected serious adverse reaction

TNFα: tumor necrosis factor-alpha

VAP: ventilator-associated pneumonia

VEGF: Vascular endothelial growth factor

STUDY SYNOPSIS

Background	High mortality associated with sepsis and MODS calls for alternative, individualized therapies in selected patients that might benefit form specific interventions. Role of macrolides as potential immunomodulatory treatment in sepsis is promising, but unclear. Subgroup analysis of previous large-scale clinical trials on patients with ventilator-associated pneumonia or gram-negative sepsis, showed that addition of clarithromycin to standard antibiotic therapy conferred a significant survival benefit in the subgroup of patients with respiratory dysfunction and MODS, but this effect has never been investigated through randomized controlled trials in an entire population suffering from these entities.
Aim	The study is aiming to assess the efficacy of intravenous treatment of clarithromycin in the reduction of 28-day mortality among patients with sepsis and respiratory dysfunction. Secondary aims are the effect on 90-day mortality, sepsis resolution, and recurrence, mortality in the subgroup of patients with septic shock and finally, impact on biomarkers of sepsis-induced immunosuppression
Design	Multicenter, interventional, double blind, randomized, placebo-controlled phase IV study
Inclusion criteria	1. Adult patients (≥18 years) 2. Patients of both genders 3. Informed consent form signed by patient or by first-degree relative in case of patient unable to consent 4. Negative (blood or urinary) pregnancy test for female patients of reproductive age 5. Willingness to receive contraception during and seven days after the administration of the study drug. 6. Presence of one or more of the following infections: hospital-acquired pneumonia (HAP), health-care associated pneumonia (HCAP), ventilator-associated pneumonia (VAP), primary Gram-negative bacteremia and intra-abdominal infections. Definitions for these infections are given below. 7. Presence of sepsis as defined by the Sepsis-3 classification criteria[3] 8. Respiratory dysfunction defined as one PaO_2/FiO_2 ratio below 200 9. Total SOFA points for organ dysfunctions other than the respiratory function more than 3.

Exclusion criteria	1. Denial for informed consent 2. Age inferior to 18 years 3. Pregnancy (confirmed by blood or urinary pregnancy test) or lactation for female patients of reproductive age. 4. Unwillingness to receive contraception during and seven days after the administration of the study drug. 5. HIV infection (with known CD4 cell count ≤ 200/mm^3) 6. Solid organ, or bone marrow transplantation 7. Corticosteroid oral or intravenous intake greater than 0.4 mg/kg of equivalent prednisone daily over the last 15 days, or other immunosuppressive therapy 8. Known active neoplasms or other conditions unrelated to sepsis, that are compromising short-term survival (1 month) 9. Neutropenia <1000/mm^3 10. Known allergy to macrolides 11. Previous participation in the study 12. Administration of a macrolide for the current infection
Study groups	Blinded 1:1 allocation to one of the following: • *Placebo*; patients receive water for injection at a volume of 20ml diluted to a final volume of 250 ml dextrose in water 5%. This is infused once daily within 1 hour for four consecutive days. • *Active drug*; patients receive 1g of clarithromycin dissolved into 20 ml water for injection and then diluted to a final volume of 250 ml dextrose in water 5%. This is infused once daily within 1 hour for four consecutive days as previously described. All patients will also receive standard therapy for sepsis, at the discretion of their attending physicians.
Primary study endpoint	To assess the impact of intravenously administered clarithromycin as adjunctive treatment to standard antibiotic therapy compared to placebo on all-cause 28-day mortality.
Secondary study endpoints	To assess the effect of clarithromycin treatment compared to placebo treatment on the following: • 28-day mortality in the subgroup of patients with septic shock • All-cause 90-day mortality

	• Early sepsis response, defined by an at least 25% decrease of day 1 SOFA score on day 3 • Sepsis response; this is defined by an at least 25% decrease of day 1 SOFA score on day 7 • New sepsis episode until day 28. A new sepsis episode is noted in any patient who experiences at least 25% decrease of day 1 SOFA score on day 7 and who has further increase of day 7 total SOFA by at least 2 points, consequent to infection • Time until new sepsis episode. A new sepsis episode is noted in any patient who experiences at least 25% decrease of day 1 SOFA score on day 7 and who has further increase of day 7 total SOFA by at least 2 points, consequent to infection • Biomarkers of sepsis-induced immunosuppression through genome, transcriptome, metabolome, microbiome and cell population analysis • Cost-effectiveness analysis of clarithromycin against placebo in the study population
Sample size calculation	This is done for the primary study endpoint. We are planning a study of independent cases and controls with 1 control per case. Prior data indicate that 28-day mortality among placebo-treated patients with sepsis and respiratory dysfunction was 55% and that this was decreased to 30% in patients receiving clarithromycin. We need to study 55 subjects into each group to be able to reject the null hypothesis that the failure rates for both groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.10. We will use a continuity-corrected chi-squared statistic or Fisher's exact test to evaluate this null hypothesis.

INTRODUCTION

Sepsis is a condition with actually rising incidence, estimated around 19 cases per 1000 hospitalizations per year in academic hospitals in USA[1] and similar trends in Europe[2]. It is associated with unacceptably high early (in-hospital) mortality of 40-50%[3]. Current guidelines promote best practice by early recognition and management with timely antibiotic administration, fluids, vasopressors and early identification/ control of infection source[4]. However, in spite of adherence to more intensive and costly protocols of early goal-directed therapy, no further decrease in mortality is achieved[5]. Syndromic approaches on sepsis and therapies targeting immune modulation are under evaluation and failures may partly be due to incomplete understanding of underlying pathophysiological mechanisms and immunological phases (pro and anti-inflammatory) of sepsis[6].

Macrolides, such as clarithromycin or azithromycin have been shown beneficial in reducing COPD exacerbations (OR 0.55; 95% CI 0.39-0.77; p<0.001) partly through anti-inflammatory properties[7]. Besides, when added to a beta-lactam regimen in community-acquired pneumonia, macrolide therapy was associated with reduced mortality risk (OR 0.67; 95% CI 0.61-0.73; p<0.001) in a recent meta-analysis of observational studies including 42942 patients[8]. An immunomodulatory effect of macrolides, beyond their antimicrobial action may explain these findings.

Clarithromycin as adjunctive treatment to standard antibiotic therapy has been used by our group in two previous large-scale multi-center RCTs conducted in Greece after approval from the Greek regulatory authorities. The first RCT studied 200 patients with sepsis due to VAP and it was conducted between 2004 and 2005 (www.clinicaltrials.gov NCT00297674). Identified pathogens mainly included gram-negative bacteria i.e. *Acinetobacter baumanii* and *Pseudomonas aeruginosa* that do not belong to the usual antimicrobial spectrum of macrolides. Although 28-day mortality in both arms was similar, patients assigned to the clarithromycin arm experienced earlier weaning from mechanical ventilation and more rapid resolution of VAP, compared to the placebo arm. In the subgroup of patients with septic shock and multiple organ dysfunction syndrome (MODS), probability of sepsis-related death was significantly lower (OR 3.78 vs 19; p = 0.043). Serious adverse events (SAEs) occurred in 3 (3%) clarithromycin-treated patients, with no clear causative link with the study drug in 2 out of 3 cases[9]. Furthermore, hospitalization costs associated with

clarithromycin use were significantly reduced (by 7000 euros/ patient that remained alive) compared to the placebo arm[10].

The second multi-center RCT compared the efficacy of clarithromycin versus placebo added to standard antibiotic therapy in patients with gram-negative sepsis caused by acute pyelonephritis, intra-abdominal infections and primary gram-negative bacteremia; 600 patients were included between 2007 and 2011 (www.clinicaltrials.gov NCT01223690). Overall mortality at 28 days in both arms did not differ, but probability of death due to septic shock and MODS was shown once more lower in the clarithromycin-treated group (OR 3.58 vs 6.21; p = 0.036). Interestingly, survival benefit from clarithromycin was even greater in those patients suffering from adult respiratory distress syndrome (ARDS). SAEs were described in 2 (0.7%) patients treated with clarithromycin, while its use was associated with saving of a median of 1000 euros/ hospitalized patient[11]. No QT space prolongation or any arrhythmia was observed in both aforementioned trials.

Immunomodulatory effects of clarithromycin in sepsis are not yet elucidated. The analysis of circulating monocytes and of circulating cytokines of patients participating in the first RCT showed that treatment with clarithromycin was associated with a decline in IL-10/ TNFα ratio, greater apoptosis of monocytes, enhanced antigen presentation capacity of monocytes, as well as improved capacity of monocytes for cytokine production suggesting an effect consistent with reversal of sepsis-induced immunosuppression[12]. These findings in conjunction with evidence generated from in vitro and animal experiments suggest modulation of the immune response as the mechanism of action of clarithromycin[13, 14].

In both RCTs, clarithromycin was administered intravenously at a dose of 1g as continuous one-hour intravenous infusion for three or four days. The drug was safe, well tolerated and cost-effective. However, benefit from treatment in both RCTs was shown only after sub-group analysis of the sub-group of patients with respiratory dysfunction and not when analysis comprised the entire study population. As a consequence, in order to consolidate the benefit of clarithromycin among patients with sepsis and respiratory dysfunction, an RCT is needed in a patient population with sepsis and respiratory dysfunction. This RCT should also comprise a study population with infections likely to be caused by Gram-negative bacteria that do not belong to the antimicrobial spectrum of clarithromycin.

AIM OF THE STUDY

The study is aiming to prove the efficacy of intravenous treatment of clarithromycin in the reduction of 28-day mortality among patients with sepsis and respiratory dysfunction. Secondary aims are the effect on overall 90-day mortality, sepsis resolution and recurrence, 28-day mortality in the subgroup of patients with septic shock and impact on genomic, transcriptomic, metabolomic, microbiome and cell population profile, especially biomarkers of sepsis-induced immunosuppression. Finally, this study aims to perform a cost-effectiveness analysis of clarithromycin compared to placebo at 90 days.

STUDY DESIGN

Type of study and study sites

This is a double- blind, randomized, placebo-controlled clinical study that will be conducted in patients admitted in the following departments of Intensive Care Medicine (ICU) and Internal Medicine in Greece and Belgium (see Appendix I):

4th Department of Internal Medicine, ATTIKON University Hospital, Athens, Greece

2nd Department of Critical Care Medicine, ATTIKON University Hospital, Athens, Greece

Intensive Care Unit, THEAGENEION General Hospital, Thessaloniki, Greece

Intensive Care Unit, G. GENNIMATAS General Hospital, Thessaloniki, Greece

Intensive Care Unit, O AGHIOS DIMITRIOS General Hospital, Thessaloniki, Greece

Intensive Care Unit, HIPPOKRATION General Hospital, Thessaloniki, Greece

Intensive Care Unit, KORGIALENEIO BENAKEIO General Hospital, Athens, Greece

Intensive Care Unit, LAIKO General Hospital, Athens, Greece

2nd Department of Internal Medicine, SISMANOGLEION General Hospital, Athens, Greece

Department of Intensive Care, ERASME University Hospital, Brussels, Belgium

Department of Intensive Care, Horta Site, BRUGMANN Hospital, Brussels, Belgium

Department of Intensive Care, Brien Site, BRUGMANN Hospital, Brussels, Belgium

Department of Intensive Care, SAINT-PIERRE Hospital, Brussels, Belgium

This is a phase IV RCT and the study drug will be provided by the Sponsor. The study protocol will be submitted for approval to the Institutional Review Board and subsequently to the Regulatory Authorities of each country; these are the National Ethics Committee and the National Organization of Medicine of Greece or the Federal Agency of Drugs and Medicinal Products of Belgium. After study approval and before enrolment of the first patient, the study will be registered at the website www.clinicaltrials.gov. Patients will be enrolled after written informed consent provided by themselves or by legal representatives (first-degree relatives) in case of patients unable to consent.

Inclusion criteria

ALL following criteria should be met for the inclusion of a patient in the study:

- Adult patients (≥18 years)
- Patients of both genders
- Informed consent form signed by patient or by first-degree relative in case of patient unable to consent
- Negative (blood or urinary) pregnancy test for female patients of reproductive age
- Willingness to receive contraception during and seven days after the administration of the study drug.
- Presence of one or more of the following infections: hospital-acquired pneumonia (HAP), health-care associated pneumonia (HCAP), ventilator-associated pneumonia (VAP), primary Gram-negative bacteremia or intra-abdominal infections. Definitions for these infections are given below.
- Presence of sepsis as defined by the Sepsis-3 classification criteria[3] (see definitions section below)
- Respiratory dysfunction defined as one PaO_2/FiO_2 ratio inferior to 200, independently of the PEEP level.
- Total SOFA points for organ dysfunctions other than the respiratory function more than 3

Exclusion criteria

Patients who meet ANY of the exclusion criteria below cannot be enrolled in the study:

- Denial for informed consent
- Age inferior to 18 years
- Pregnancy (confirmed by blood or urinary pregnancy test) or lactation for female patients of reproductive age.
- Unwillingness to receive contraception during and seven days after the administration of the study drug.
- HIV infection (with known CD4 cell count ≤ 200/mm^3)
- Solid organ, or bone marrow transplantation
- Corticosteroid oral or intravenous intake greater than 0.4 mg/kg of equivalent prednisone daily over the last 15 days; anti-cytokine biological agents (e.g. anti-TNFα), anti-lymphocyte immunoglobulins, Mycofenolate Mofetil, Tacrolimus (FK506) and m-TOR inhibitors (any dose of the above within the past 3 months); Chemotherapy within the past 3 months, Leflunomide intake within the past 2 year, Rituximab within the last year; Methotrexate, Azathioprine, Cyclosporine, Cyclophosphamide (any dose of the above within the last 3 months). Splenectomy, known primary immunodeficiencies. Hydroxyurea, anti-Vascular Endothelial Growth Factor (VEGF), anti-Epithelial Growth Factor Receptor (EGFR), anti-Growth Factor Her-2 and Interferon a, b and γ intake are not considered as exclusion criteria.
- Known active neoplasms or other medical conditions unrelated to sepsis (any of the two), that are compromising short-term survival (1 month)
- Neutropenia <1000/mm^3
- Known allergy to macrolides
- Previous participation in the study
- Administration of a macrolide for the current infectious episode

Definitions

Sepsis is defined by the following criteria, based on sepsis-3 classification[3]:

- Total SOFA score of 2 or more points for patients who are admitted with infection at the emergency department (see APPENDIX II) or

- Increase of admission SOFA score by 2 or more points consequent to infection, for patients already hospitalized (see APPENDIX II)

Hospital-acquired pneumonia (HAP) is defined by the presence of a new or progressive radiographic lung infiltrate in a non-intubated patient hospitalized for more than 48 hours who presents with at least two of the following clinical features:

- Core temperature equal or greater than 38°C
- Total white blood cell count more than 12,000/mm^3
- Rales or bronchial breath sounds on physical examination
- Purulent sputum
- More than 20 breaths/minute
- Serum procalcitonin more than 0.25 ng/ml
- Gram stain of tracheobronchial secretions or bronchoalveolar lavage fluid indicating the predominance of Gram-negative bacilli

Health-care associated pneumonia (HCAP) is defined by the presence of a new or progressive radiographic lung infiltrate in a non-intubated patient who has at least one of the following risk factors for HCAP[15]:

- Hospitalization the last 90 days
- Residency in a long-term care facility
- Under regular hemodialysis

AND who presents with at least two of the following clinical features:

- Core temperature equal or greater than 38°C
- Total white blood cell count more than 12,000/mm^3
- Rales or bronchial breath sounds on physical examination
- Purulent sputum
- More than 20 breaths/minute
- Serum procalcitonin more than 0.25 ng/ml
- Gram stain of tracheobronchial secretions or bronchoalveolar lavage fluid indicating the predominance of Gram-negative bacilli[15]

Ventilator-associated pneumonia (VAP) is defined by the presence of a new or progressive radiographic lung infiltrate in a patient who is under mechanical

ventilation for at least 48 hours AND who presents with at least two of the following clinical features:

- Core temperature equal or greater than 38°C
- Total white blood cell count more than 12,000/mm^3
- Purulent tracheobronchial secretions
- Serum procalcitonin more than 0.25 ng/ml
- Gram stain of tracheobronchial secretions or bronchoalveolar lavage fluid indicating the predominance of Gram-negative bacilli[15]

Primary Gram-negative bacteremia (BSI) is defined as the isolation of at least one Gram-negative microorganism from a blood culture of a peripheral vein of a patient that is not related to infection of a central line and who presents with ALL the following features[16]:

- Core temperature equal or greater than 38°C or total white blood cell count more than 12,000/mm^3
- Thorough clinical and radiological investigation has failed to identify the primary infection site

Intra-abdominal infection (IAI) is defined as the presence of ALL the following features[16]:

- Core temperature equal or greater than 38°C or total white blood cell count more than 12,000/mm^3
- Radiological findings from abdominal ultrasound or abdominal computed tomography or magnetic resonance imaging consistent with one IAI or peri-operative confirmation of an IAI.

Study drug preparation and administration

A separate allocation sequence will be generated for each study site, following a 1:1 design. An allocation sequence will be generated as sealed envelope from a statistician with 1:1 randomization per study site. The envelope is unsealed by an unblinded investigator (or the pharmacist), who is preparing the study drug (placebo or active drug). The preparations will be visually similar and allow blinded administration. Clarithromycin is provided in a form of vial with 500mg of amorphous

powder. Two vials are dissolved in 10ml of water for injection each. The prepared 20ml solution is then further diluted to a final volume of 250 ml dextrose in water 5% that is directly connected to the infusion device that leading to a catheter already inserted in a central or peripheral vein. Placebo will consist in 20 ml of water for injection, diluted to a final volume of 250 ml dextrose in water 5% that is directly connected to the infusion device leading to a catheter already inserted in a central or peripheral vein. According to the generated allocation sequence, patients of each study site can be randomly assigned to one of the following two groups:

- *Placebo*; patients receive water for injection at a volume of 20ml diluted to a final volume of 250 ml dextrose in water 5%. This is infused once daily within 1 hour for four consecutive days. All patients allocated to the placebo group will also receive standard therapy at the discretion of their attending physicians.
- *Active drug*; patients receive 1g of clarithromycin dissolved into 20 ml water for injection and then diluted to a final volume of 250 ml dextrose in water 5%. This is infused once daily within 1 hour for four consecutive days as previously described[9, 11]. All patients allocated to the active drug group will also receive standard therapy at the discretion of their attending physicians.

Study visits

Screening visit

When a patient meets ALL inclusion criteria and NONE of the exclusion criteria, he/she can be enrolled in the study. The criteria are judged based on data available from the patient's file. Female patients of reproductive age should be screened with a urinary pregnancy test. QT prolongation or prior arrhythmia is not considered as an exclusion criterion for the study, [9,11] but may be considered in the decision making of the principal investigator. The same patient cannot be enrolled twice in this study.

Patient follow-up

Follow-up of every patient will be done daily until day 28 or hospital discharge (whatever comes first).

Visit 1 is on day 1. Procedures of this day include:

- Recording of demographics, medical history, co-morbidities (Charlson Comorbidity Index), [17] SOFA score (see APPENDIX II); Acute Physiology and Chronic Health Evaluation (APACHE) II score (see APPENDIX III), available blood cell count, biochemistry, coagulation time, urine output, blood gas analysis, recording of suspected infection site, available radiological findings, relevant for the current infection microbiology and antimicrobial susceptibility testing if available, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Sampling of 35 ml of venous blood; 3ml is collected into one PAXgene tube or a tube with RNA*later*®; 9ml is collected into one pyrogen-free tube; and 24ml is collected into EDTA-coated tubes
- Collection of stool culture or rectal swab
- Administration of the study drug
- Evaluation of potential adverse events

Visit 2 is on day 2. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, relevant for the current infection microbiology and antimicrobial susceptibility testing if available, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Administration of the study drug
- Evaluation of potential adverse events

Visit 3 is on day 3. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, relevant for the current infection microbiology and antimicrobial susceptibility testing if available, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.

- Evaluation of early sepsis response, defined by at least 25% decrease of visit 1 SOFA on visit 3
- Survival status
- Administration of the study drug
- Evaluation of potential adverse events

Visit 4 is on day 4. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, recording of suspected or proven infection site, available radiological findings, relevant for the current infection microbiology and antimicrobial susceptibility testing if available, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Administration of the study drug
- Evaluation of potential adverse events

Visit 5 is on day 5. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, relevant microbiology and antimicrobial susceptibility testing if available, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation. Given the previous data from phase IV trials, serial ECGs are not required for this protocol.
- Survival status
- Sampling of 35 ml of venous blood; 3ml is collected into one PAXgene tube or a tube with RNA*later*®; 9ml is collected into one pyrogen-free tube; and 24ml is collected into EDTA-coated tubes
- Collection of stool or rectal swab.
- Evaluation of resolution of the infection and potential recurrence of infection according to the judgment of the attending physician
- Evaluation of potential adverse events

Visit 6 is on day 6. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, clinically relevant microbiology and antimicrobial susceptibility testing, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Evaluation of resolution of the infection and potential recurrence of infection according to the judgment of the attending physician
- Evaluation of potential adverse events

Visit 7 is on day 7. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, clinically relevant microbiology and antimicrobial susceptibility testing, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Evaluation of sepsis response, defined by at least 25% decrease of SOFA score of visit 1
- Evaluation of resolution of the infection and potential recurrence of infection according to the judgment of the attending physician
- Evaluation of potential adverse events

Visit 8 is on day 8. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, clinically relevant microbiology and antimicrobial susceptibility testing, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.

- Survival status
- Evaluation of resolution of the infection and potential recurrence of infection according to the judgment of the attending physician
- Evaluation of potential adverse events

Visit 9 is on day 9. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gas analysis, available radiological findings, clinically relevant microbiology and antimicrobial susceptibility testing, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Evaluation of resolution of the infection and potential recurrence of infection according to the judgment of the attending physician
- Evaluation of potential adverse events

Visit 10 is on day 10. Procedures of this day include:

- Recording of SOFA score (see APPENDIX II); available blood cell count, biochemistry, coagulation times, urine output, blood gases, available radiological findings, clinically relevant microbiology and antimicrobial susceptibility testing, administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Evaluation of resolution of the infection and potential recurrence of infection according to the attending physician
- Sampling of 33 ml of venous blood; 9ml is collected into one pyrogen-free tube; and 24ml is collected into EDTA-coated tubes
- Evaluation of potential adverse events

Visits 11-28 are on days 11-28 respectively. They include

- Recording of SOFA score if available

- Administered antimicrobials, other administered drugs and need for source infection control either by percutaneous interventions or by any operation.
- Survival status
- Evaluation of resolution of the infection and potential infection recurrence according to the judgment of the attending physician
- Evaluation of potential adverse events

If the patient is discharged before day 28, a phone call will be performed on day 28 to assess survival status, need for new hospitalization or antibiotic therapy and potential adverse events. End-of-life decisions, if any, will be recorded.

Last visit: A phone call follow-up will be done on day 90 to assess survival status. If available, data concerning any new hospitalization, or antibiotic cure and potential adverse events will be collected during that phone call. Moreover, a self or proxy-assessment of health-related quality of life will be performed through the EQ-5D-3L™ questionnaire on the same visit (see APPENDIX IV) [18].

All information will be recorded on a specific paper Case Report Form (CRF).
The procedure to follow at each study visit is shown in APPENDIX V.

Laboratory analysis

Collected samples will be transported to the central lab that is the Laboratory of Immunology and Infectious Diseases of the 4th Department of Internal Medicine, ATTIKON University General Hospital, Athens, Greece. Analysis will be done as follows:

- PAXgene tubes or tubes with RNA*later*® for will be stored in -80°C for full transcriptomics
- EDTA tubes will be used: partly for direct isolation of peripheral blood mononuclear cells and further cytokine stimulation, partly for flow cytometry, and partly for whole blood immune functional assay. The remaining blood in EDTA tubes will be centrifuged and plasma will be stored in -20°C for metabolomics, cytokine analysis with enzyme immunoassay (EIA) and antibiotic concentration measurement with high-performance liquid chromatography (HPLC) in patients receiving meropenem, tigecyclin or colistin; leucocyte buffy coat remaining after

plasma aspiration with sterile pipette will be equally stored in -20°C for genome analysis.

- Serum isolated from pyrogen and anticoagulant-free tube after centrifugation will be stored in -20°C to be used for metobolomic analysis and serum markers.
- Stool or rectal swab will be stored in -80°C, to be later processed for 16S rRNA sequencing of the intestinal microbiome.

Cost analysis

The cost of hospitalization associated with each intervention will be calculated in two ways, both of them from a National Health Service and personal social services perspective:

1) According to the first way, cost per day will be defined by the sum of multiplications of each counted item or resource (including administered antimicrobials, other drugs, fluids, blood products, any invasive procedure for source control, mechanical ventilation, renal replacement therapy, radiological examinations, insulin administered for standard glycemic control, enteral or parenteral nutrition) with its price in Euros (€) and the addition of the nominal cost of daily stay for the ICU or general ward. The unit price for each counted item will derive from the National Health Service tariff data official pricelist. The following are excluded from the analysis as their price is considered negligible and/ or equally distributed among groups: vitamins, reconstitution/ dilution fluids, electrolytes, catheter insertions, perfusion devices, monitoring devices, consumables such as laboratory testing, cleaning packs, gloves, or masks, echocardiography performed in ICU for monitoring purposes, transport costs at discharge, cost of human resources such as salary of nursing or medical personnel. Counting of the items will be performed from enrollment until discharge or day 28, by investigators completely blind to the allocated treatment.
2) According to the second way, the total cost of the index hospitalization (where enrollment took place) will be extracted from administrative hospital and insurance records, which are based on Diagnosis Related Groups (DRGs), a combination of ICD-10 diagnosis and codes of medical acts. This is completed by any additional cost of medication or

medical equipment plus any extra bed-days per medical or surgical ward or ICU stay, for medications and bed-days exceeding the estimated length of stay per DRG.

Direct cost of additional medical care (e.g. new hospitalizations) up to 90 days will be recorded, if available.

Cost-Effectiveness analysis

The EuroCoL EQ-5D-3L™ questionnaire at 90 days (proxy and telephone version) data will be used to convert patient responses into Quality-Adjusted Life-Years (QALYs), to define patient-level perception of health-related quality of life on a 0,1 scale. Deceased patients will be counted as a 0 on that scale. Quality of life will be compared with the cost of hospitalization.

STUDY ENDPOINTS

Primary study endpoint

To assess the impact of intravenously administered clarithromycin as adjunctive treatment to standard antibiotic therapy compared to placebo on all-cause 28-day mortality.

Secondary study endpoints

To assess the effect of clarithromycin treatment compared to placebo treatment on the following:

- 28-day mortality in the subgroup of patients with septic shock
- All-cause 90-day mortality
- Early sepsis response, defined by an at least 25% decrease of day 1 SOFA score on day 3
- Sepsis resolution; this is defined by an at least 25% decrease of day 1 SOFA score on day 7
- New sepsis episode until day 28. A new sepsis episode is defined as a further increase of SOFA score by at least 2 points consequent to infection, in a patient who has experienced previous sepsis resolution (at least 25% decrease of day 1 SOFA score on day 7)

- Time until new sepsis episode. A new sepsis episode is noted in any patient who experiences any more than 25% decrease of day 1 SOFA score on day 7 and who has further increase of SOFA by at least 2 points, consequent to infection
- Biomarkers of sepsis-induced immunosuppression, through genome, metabolome, transcriptome, microbiome and cell population analysis
- The (primary) health economic outcome is cost per quality-adjusted life-year (QALY) gained for clarithromycin compared with placebo.

POWER CALCULATION

This is done for the primary study endpoint. We are planning a study of independent cases and controls with 1 control per case. Prior data indicate that the mortality among placebo-treated patients with sepsis and respiratory dysfunction was 55% and that this was decreased to 30%[11]. We need to study 55 subjects into each group to be able to reject the null hypothesis that the failure rates for both groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.10. We will use a continuity-corrected chi-squared statistic or Fisher's exact test to evaluate this null hypothesis.

STATISTICAL ANALYSIS

Qualitative endpoints will be analyzed by the Fisher's exact test or continuity corrected Chi- square test, as appropriate. Logistic regression models will be used to evaluate variables associated with 28-day mortality. The effect on sepsis-induced immunosuppression will be a composite endpoint and it will result from the bioanalysis of gene expression, circulating cytokines and stimulated cytokines of patients. More precisely, bioanalysis of gene expression is anticipated to provide pathways modulated by treatment. These pathways will be validated by measurement of representative cytokines in cell supernatants. Comparisons of over-time changes will be done between the two groups of treatment. A p - value lower than 0.05 will be considered statistically significant, unless stated otherwise.

For the cost analysis, cost of each intervention will be counted and compared between groups, with non-parametric Mann-Whitney test, as previously described[10]. This analysis will be subject to robustness control to assess potential differences between different methods of cost calculation. Cost-effectiveness will be expressed as an incremental cost-to-effectiveness ratio (ICER) of clarithromycin compared with

placebo, in a within-trial analysis (90 days). The ICER will be compared to a willingness-to-pay factor, which is either defined by the National Institute for Health and Care Excellence threshold or by per capita Gross Domestic Product. [19, 20]
To evaluate the variation and significance of the ICER estimate, a bootstrapping method will be used.

DURATION OF THE STUDY

The duration of the study is estimated to two years after approval by the Greek and Belgian regulatory authorities.

ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient's last evaluation. An adverse event is any undesirable medical occurrence in a subject receiving a pharmaceutical product and which does not necessarily have a causal link with this treatment. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

An adverse reaction is any undesirable and unintended reaction due to investigational medicine product administration (or intervention), related with any dose administrated. If an adverse event/ reaction meets any of the following criteria, it is considered as a *Serious Adverse Event/ Reaction* (SAE):

- Death
- Life-threatening situation The subject was at risk of death at the time of the adverse event/ experience. It does not refer to the hypothetical risk of death if the AE/ adverse reaction were more severe or were to progress.
- Hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/ incapacity Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- Congenital anomaly/ birth defects Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- Important medical events/ experiences that may not result in death, be life-threatening, or require hospitalization, may be considered as SAE when, based

upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above, i.e. death, a life-threatening adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/ incapacity, or a congenital abnormality/ birth defect. Examples of such medical events/ experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

- Spontaneous and elective abortions experienced by study subject

A non-serious adverse event is one that does not meet the previous definition of a SAE. The severity of the non-serious adverse events will be graded using the following definitions:

- *Mild* - the adverse event/ reaction is transient and well tolerated by the patient
- *Moderate* – The adverse event/ reaction causes discomfort and affects the usual activities of the patient.
- *Severe* – The adverse event/ reaction affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

Relationship with the drug

The time relationship is established if the AE occurs during therapeutic treatment and until 5 half-lives after treatment discontinuation. The investigator will use the following definitions to assess the relationship of the adverse events with the study drug:

- Probably Related: The adverse event has a strong time relationship with the drug or relapses if re-induced, and another etiology is improbable or clearly less probable.
- Possibly Related: The adverse event has a strong time relationship to the drug and an alternative etiology is as probable or less probable.
- Probably not Related: The adverse event has a slight or no time relationship to the drug and/or there is a more probable alternative etiology.

- Unrelated: The adverse event is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative etiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternative etiology must be provided by the investigator. Please note that a severe adverse event/ experience is not necessarily serious, as the term severe is a measure of intensity, while a serious adverse event is determined based on the aforementioned regulatory criteria.
Individual un-blinding thought to be necessary for the management of an adverse event will be documented in the subject CRF.

All Investigators are held to report every adverse event and evaluate the severity and possible causality with the study drug according to aforementioned criteria. All adverse events/ reactions are reported to Sponsor. The sponsor is responsible for the evaluation of all AEs. All Serious Adverse Events/ Serious Adverse Reactions must be reported to the Sponsor within 24 hours after having the information, by completion of the SAE form and fax to Hellenic Institute of Sepsis. Untoward events resulting from sepsis or infection (such as death or non-resolving infection) will not be recorded as AEs or SAEs, since they are study endpoints.

The Sponsor must evaluate whether an adverse event is expected or not. A SAE may qualify for expedited reporting to regulatory authorities if it is determined to be a suspected, unexpected serious adverse reaction (SUSAR- an adverse reaction, the nature or severity of which is not consistent with the applicable product information -e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product). The Sponsor is responsible for submitting expedited safety reports to the appropriate regulatory authority and ethics committee for all confirmed SUSARs. In the case of a fatal or life-threatening SUSAR, the Sponsor will notify the regulatory authorities and the ethics committee as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. For a non-life-threatening SUSAR, the report will be submitted no later than 15 days after the Sponsor is made aware of the event.

The Sponsor has the obligation to submit annually a drug safety updated report (DSUR) according to global experience to the appropriate regulatory

authorities. The electronic submission to Eudravigilance will be performed through the Organization ID: HISS.

The above pharmacovigilance procedures will be performed on behalf of the Sponsor (Hellenic Institute for the Study of Sepsis) by the Consultant Company «SUSTCHEM Engineering P.Braimiotis-P. Scarlatos LTD», 144 3rd Septemvriou str, 11251, Athens, and the Qualified Person for Pharmacovigilance (QPPV) will be Mrs Areti Voulomenou. (contact details in Appendix I).

QUALITY CONTROL AND ASSURANCE

Quality control and assurance checks are performed by the Sponsor in order to allow periodic review of adequacy of the study activities and practices and allow for revising of those practices, as needed, so the data and process are maintained, the study meets the protocol and procedural requirements, and is reproducible. Before enrolling any subject in this study, sponsor personnel and the investigator review the protocol, the Investigator's Brochure, the CRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs.

A qualified representative of the sponsor monitors the conduct of the study by visiting the site and by conduct of the study by visiting the site and by contacting the site by telephone and e-mail. During these site visits, all source documents are reviewed and information recorded in the CRFs is verified against them.

Beside routine monitoring quality assurance will be documented through independent auditing of the quality control activities and where applicable, by regulatory authorities through inspections.

ETHICAL CONSIDERATIONS

Prior to the initiation of this study, the study design will receive ethical, scientific and regulatory review. Investigators will conduct this study in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice, and applicable regulatory requirements.

Regarding informed consent form (ICF) signature, before any procedures specified in the protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent

- Be given time to ask questions and to consider the decision to participate
- Voluntarily agree to participate in the study
- Sign and date the updated and approved by ethics committee and regulatory authorities ICF version.

PROTOCOL ADHERENCE AND AMENDMENTS

Investigators ascertain that they will apply due diligence to avoid protocol deviations. All significant protocol deviations will be recorded and reported in the clinical study report (CSR). Any change or addition to the protocol can only be made in a written protocol amendment that must be approved and signed by the sponsor, investigator, ethics committee and, where required, regulatory authorities.

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APPENDIX I Participating Sites

4th Department of Internal Medicine, ATTIKON University Hospital, Athens, Greece

2nd Department of Critical Care Medicine, ATTIKON University Hospital, Athens, Greece

Intensive Care Unit, THEAGENEION General Hospital, Thessaloniki, Greece

Intensive Care Unit, G. GENNIMATAS General Hospital, Thessaloniki, Greece

Intensive Care Unit, AGHIOS DIMITRIOS General Hospital, Thessaloniki, Greece

Intensive Care Unit, HIPPOKRATION General Hospital, Thessaloniki, Greece

Intensive Care Unit, KORGIALENEIO BENAKEIO General Hospital, Athens, Greece

Intensive Care Unit, LAIKO General Hospital, Athens, Greece

2nd Department of Internal Medicine, SISMANOGLEION General Hospital, Athens, Greece

Department of Intensive Care, ERASME University Hospital, Brussels, Belgium

Department of Intensive Care, Horta Site, BRUGMANN Hospital, Brussels, Belgium

Department of Intensive Care, Brien Site, BRUGMANN Hospital, Brussels, Belgium

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Monitor of the study as assigned by the Sponsor is:

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QPPV of the study as assigned by the Sponsor is Mrs Areti Voulomenou, MEng, MSc

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APPENDIX II The SOFA score[3]

Variable	**0 points**	**1 point**	**2 points**	**3 points**	**4 points**
PaO_2/FiO_2(mmHg)	≥400	<400	<300	<200	<100
Platelets (per mm^3)	≥150	<150	<100	<50	<20
Hypotension	MAP≥ 70 mmHg	MAP<70 mmHg	Dobutamine whatever dose	Adrenaline ≤0.1* or Noradrenaline≤ 0.1*	Adrenaline>0.1* or Noradrenaline >0.1*
Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Creatinine (mg/dl) or Urine output	<1.2	1.2-1.9	2.0-3.4	35-4.9 or <500ml/day	≥5.0 or <200ml/day

*µg/kg/min

Each variable is scored between 0 and 4. The SOFA score is the sum of the score of each variable

APPENDIX III Calculation of the APACHE (acute physiology and chronic health evaluation) II score.

PARAMETER	VALUES ABOVE NORMAL				NORMAL	VALUES BELOW NORMAL			
	+4	+3	+2	+1	0	+1	+2	+3	+4
1. Rectal temperature (ºC)	>41	39-40.9		38. 5-38. 9	36-38.4	34-35.9	32-33.9	30-31.9	<29.9
2. Mean arterial pressure (mm Hg)	>160	130-159	110-129		70-109		50-69		<49
3. Heart ventricular rate	>180	140-179	110-139		70-109		55-69	40-54	<39
4. Respiratory rate (mechanical or no ventilation)	>50	35-49		25-34	12-24	10-11	6-9		<5
5. Oxygenation: $AaDO_2$ or PaO_2 (mmHg)									
α) FiO_2>0. 5: calculate $AaDO_2$	>500	350-499	200-349		<200				
β) FiO_2<0. 5: only PaO_2					PaO_2>70	PaO_2 61-70		PaO_2 55-60	PaO_2 <55
6. Arterial pH	>7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7. 15
7. Serum sodium (mMol/L)	>180	160-179	155-159	150-154	130-149		120-129	110-119	<110
8. Serum potassium (mMol/L)	>7	6-6.9		5.6-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
9. Serum creatinine (mg/dL) (x 2 in case of chronic renal failure)	>3.5	2-3.4	1. 5-1.9		0. 6-1.4		<0.6		
10. Hematocrit (%)	60		50-59.9	46-49.9	30-45.9		20-29.9		<20
11. White blood cells (1000/mm^3)	>40		20-399	15-19.9	3-14.9		1-2.9		<1
12. Glasgow Coma Score GCS (Scoring = 15 - GCS)	15 – GCS =								
Total Acute Physiology Score (APS)	Addition of scores for parameters 1-12 =								
HCO_3^- serum (venous blood – mMol/L)	52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	
To be used only if absent arterial gas									

Age	Score	Age	Score	Age	Score	Age	Score	Age	Score
<44	0	44-54	2	55-64	3	65-74	5	>75	6

Chronic disease score

If the patient has history of severe organ insufficiency or is immunodeficient, scoring is done as follows:

a. No surgery or emergency surgery: + 5 points

b. Post-operative patient after programmed surgery: + 2 points

TOTAL APACHE II SCORE: APS + AGE + CHRONIC DISEASE SCORE

APPENDIX IV EQ-5D-3L (UK English version)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

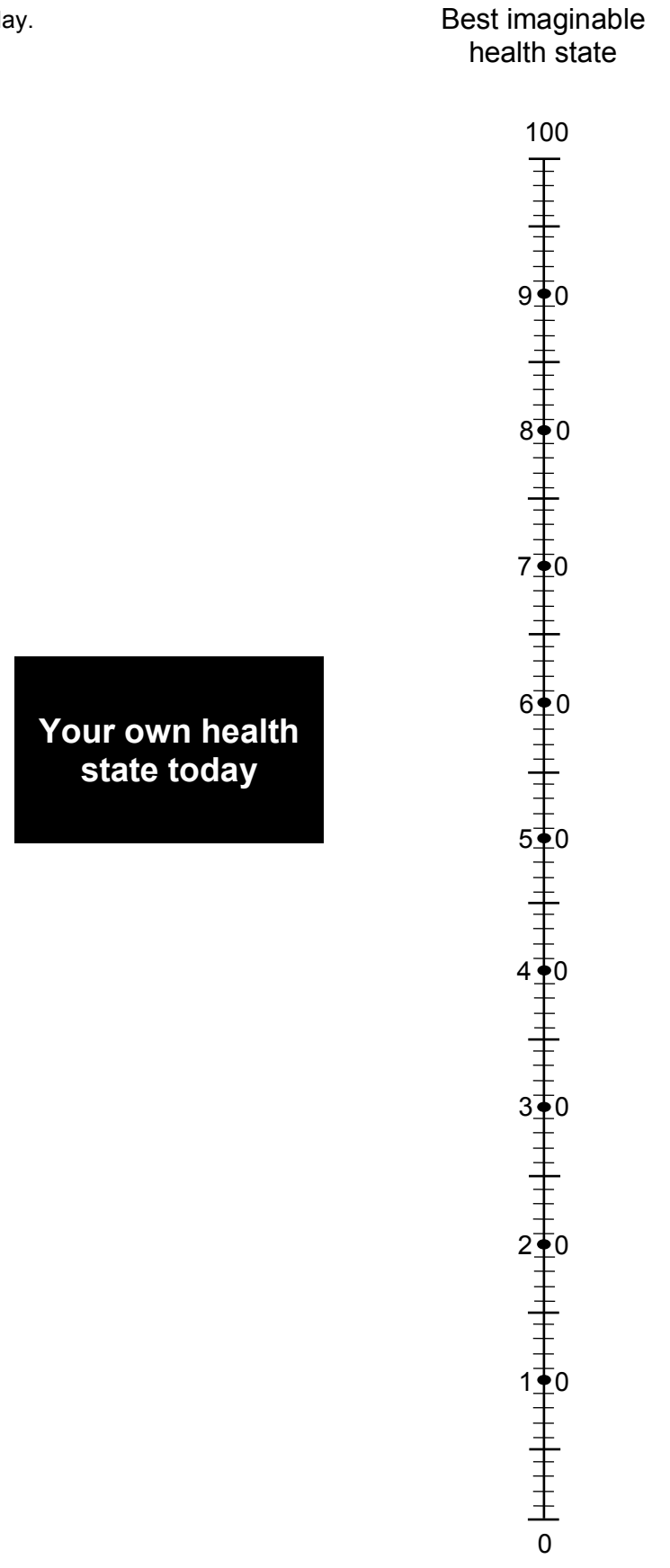
I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked **100** and the worst state you can imagine is marked **0**.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.



APPENDIX V Procedures on each study visit (until day 28 or until hospital discharge)

	Study visits																												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**Last**
Study drug	x	x	x	x																									
SOFA score*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Survival		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CCI	x																												
APACHE score	x																												
Vital signs**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Concomitant drugs/ procedures	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Infectious site identification	x			x																									
Resolution/ recurrence of infection**							x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
AE monitoring	x	x	x	x	x	x	x	x	x	x																			
Microbiology**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Pregnancy test***	x																												
PAX Gene	x				x																								
Serum metabolomics	x				x					x																			
PBMCs isolation	x				x					x																			
Stool sample	x				x																								

- * SOFA score: if available according to usual clinical practice. On days where SOFA components are not available (e.g. no blood analysis programed by the physician or discharged patient), the last known value will be recorded.
- ** If available and justified by clinical context ***For female patients of reproductive age