A RANDOMIZED PROSPECTIVE CLINICAL TRIAL TO ASSESS THE ROLE OF PROCALCITONIN-GUIDED ANTIMICROBIAL THERAPY TO REDUCE LONG-TERM INFECTIONSSEQUELAE (The PROGRESS trial)

STUDY PROTOCOL

Evangelos J. Giamarellos-Bourboulis, MD, PhD

Principal Investigator
Associate Professor of Internal Medicine
National and Kapodistrian University of Athens, Athens, Greece

EudraCt Number: 2017-002011-33
Protocol version: 3.0
Protocol phase: IV
Protocol date: 23 January 2018

DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: A RANDOMIZED PROSPECTIVE CLINICAL TRIAL TO ASSESS THE ROLE OF PROCALCITONIN-GUIDED ANTIMICROBIAL THERAPY TO REDUCE LONG-TERM INFECTIONS SEQUELAE

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 I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.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

Table of contents	**Page**

LIST OF ABBREVIATIONS

AE: adverse event

APACHE: acute physiology and chronic health evaluation

ARDS: acute respiratory distress syndrome

CDI: *Clostridium difficile* infection

CPIS: clinical pulmonary infection score

CT: computed tomography

EIA: enzyme immunoassay

HCO_3: bicarbonate

GDH: glutamate dehydrogenase

MAP: mean arterial pressure

MDR: multi drug resistant

PCT: procalcitonin

pCO_2: partial carboxide pressure

pO_2: partial oxygen pressure

pO_2/FiO_2: rate of partial oxygen pressure to the fraction of oxygen in the inspired mixture

SAE: serious adverse event

SOFA: sequential organ failure assessment

VAP: ventilator associated pneumonia,

SYNOPSIS

Aim	Early administration of antimicrobials remains the mainstay of treatment of severe infections. When microbiology cultures of biological specimens fail to provide information for the microbial cause of an infection, antimicrobial stewardship relies on the use of biomarkersand mainly procalcitonin (PCT).Procalcitoninguided-treatmentisseen to be non-inferiortothestandardantibioticapproach and leads to a shorter antibiotic exposure, having possible beneficial effect on reducing microbial resistance and therapy costs. Results of a recent study suggest that PCT guidance of antimicrobial treatment allows not only proper antimicrobial stewardship but it is also associated with survival benefit. This study did not provide, however, an explanation of the underlying mechanism of survival benefit. The aim of the study is to demonstrate if using one PCT-guided rule of stop of antimicrobials, the incidence of infections by *C.difficile* and by multidrug-resistant (MDR) bacteria during the next six months may be significantly decreased, explaining the lower mortality.
Design	Prospective, multicenter, randomized, controlled trial
Inclusion criteria	• Male or female • Age more than or equal to 18 years • Written informed consent from patient or relative • SOFA score more than or equal to 2 points for patients admitted in the emergencies and with a more than or equal to a 2-point increase of admission SOFA score for hospitalized patients. • Presence of one of the following infections: community-acquired pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia, bacteremia and acute pyelonephritis.
Study groups	• Standard of care: these patients will receive antimicrobials according to standard practice of the attending physicians but PCT will not be measured and antimicrobials will be stopped according to the local standard practice. • PCT group: these patients will receive antimicrobials according to standard practice of the attending physicians and PCT will be measured on day 1 (day of start of antimicrobials) and then daily starting from day 5 (96 hours from the start of antimicrobials). Antimicrobials will be discontinued when PCT value is less than 80% of the initial value or it remains below 0.5 ng/ml.
Primary study	The change of infection-associated adverse events rate for patients treated

endpoint	by the PCT-guided stopping rule compared to patients treated by standard of care. The infection-associated adverse events rate is considered as the advent of any case of CDI or infection by MDR or new colonization by MDR and/or *C.difficile*or infection-associated death with the first six months from study inclusion.
Secondary study endpoints	• Time to first infection-associated adverse events rate until month 6. For patients with more than one infection-associated adverse event the first one is encountered. • Rate of CDI until month 6. For patients with more than one episode of CDI the first one is encountered. • Rate of infections by MDR until month 6. For patients with more than one infection by MDR event the first one is encountered. • All-cause28-day mortality • All-cause6-month mortality • Rate stool positive for GDH by *C.difficile* until month 6 • Rate of stool colonization by MDR until month 6 • Microbiome composition on day 28 • Changes of the microbiome between days 1 and 28and between day 1 and 6 months • Consumption of antimicrobials until hospital discharge • Real cost until hospital discharge
Power of the study	The study is powered for the primary endpoint. It is anticipated that the rate of infection-associated adverse events in the standard-of-care group will be 30% and that by using the PCT-guided stop of antimicrobial it will be decreased to 15%. To achieve 80% power at the 5% significance level to demonstrate this difference, 133 patients should be enrolled in each arm as analyzed by the Power and Sample Size Calculation Program (version 3.1.2). To adjust for missing values, it is anticipated that 140 patients should be enrolled in each arm.

BACKGROUND

Early administration of antimicrobials remains the mainstay of treatment of severe infections(1,2). Current guidelines of management of severe sepsis suggest that initial therapy of a patient should be reviewed after 48 to 72 hours (2). At that stage some patients are doing well, whereas others fail to respond. When microbiology cultures of biological specimens fail to provide information for the microbial cause of an infection and susceptibilities to antimicrobials, antimicrobial stewardship relies on the use of biomarkersand mainly procalcitonin (PCT). Datasofar,suggestthat early changes of serum PCT can inform about the prognosis of the septic patient, with greater values reflecting a worse outcome and higher mortality and that serial measurements within 48-72 hours provide adequate information of the appropriateness of the administered antimicrobials (3,4). Moreovertheuseofaprocalcitoninguided-treatment in surgical (5) as well as in non-surgical (6) critically-ill patients,isseen to be non-inferiortothestandardantibioticapproach and leads to a shorter antibiotic exposure, having possible beneficial effect on reducing microbial resistance and therapy costs.

In the largest study conducted so far, de Jong et al (7) showed that PCT-guided stop of treatment was not only safe compared with standard of care antibiotic duration, but also led to a better outcome i.e. significant decrease of both 28-day and 1-year mortality. The results of this study are a major contribution in the field of critical care since they prove for the first time that PCT guidance of antimicrobial treatment allows not only proper antimicrobial stewardship but it is also associated with survival benefit. However, de Jong et al did not provide findings to explain the underlying mechanism of survival benefit. As a rule critically ill patients run two major risks coming from the long-term administration of antimicrobials; the first is infections by*Clostridium difficile*coming from the ecological damage of gut flora (8, 9) and the second is the risk of infections by multidrug-resistant (MDR) bacteria colonizing the gut (10, 11, 12). MDR is emerging after the ecological pressure of broad-spectrum antimicrobial usually administered to the critically ill patient.

AIM OF THE STUDY

The aim of the study is to demonstrate if using one PCT-guided rule of stop of antimicrobials, the incidence of infections by *C.difficile* and by MDR bacteria during the next six months may be significantly decreased.

PATIENTS AND METHODS

Study design

This a prospective, multicenter, randomized, controlled trial among patients hospitalized due to severe infections in departments participating in the Hellenic Sepsis Study Group. The study will be submitted for approval by the Ethics Committees of the participating hospitals, of the National Ethics Committee of Greece and by the National Organization for Medicines of Greece. Patients will be enrolled after written informed consent provided by themselves or provided by first-degree relatives in case of patients unable to consent.

Inclusion criteria

All enrolled patients should meet all following inclusion criteria:

- Male or female
- In case of women, unwillingness to remain pregnant during the study period.
- Age more than or equal to 18 years
- SOFA score more than or equal to 2 points for patients admitted in the emergencies and with a more than or equal to a 2-point increase of admission SOFA score for hospitalized patients. The SOFA score will be determined according toTable 1.
- Presence of one of the following infections: community-acquired pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia, bacteremia and acute pyelonephritis. Infections are defined according to Table 2. Any infection with onset more than 48 hours post hospital admission is considered one hospital-acquired infection.

Exclusion criteria

Patients meeting ANY of the following should NOT be enrolled.

- Failure to obtain written consent to participate

- Patients in pregnancy or breastfeeding.Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study
- Patients receiving prolonged antibiotic therapies (e.g. endocarditis, implantable device-associated infection, cerebral/hepatic abscess, osteomyelitis, meningitis)
- Patients with severe infections due to viruses or parasites (e.g. Dengue, *Toxoplasma gondii*, *Plasmodium* spp.)
- Patients infected with *Mycobacterium tuberculosis*.

Table 1 Variables taken into consideration for SOFA score determination

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

*µg/kg/min

Table 2 Definitionsofinfections

Infection	All the following	At least 2 of the following	At least 1 of the following
Bacteremia	At least 1 positive blood culture	None	None
Community-acquired pneumonia (14)	New or evolving infiltrate on chest X-ray	• New onset or worsening of cough • Dyspnea • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥0.25 ng/ml • Hypoxemia pO2≤60mmHg or oxygen saturation ≤90% in room air • Respiratory rate ≥20 breaths/min
Hospital-acquired pneumonia (15)	• Onset >48 hours from hospital admission • New or evolving infiltrate on chest X-ray	• New onset or worsening of cough or dyspnea • Purulent tracheobronhchial secretions • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥0.25 ng/ml • Hypoxemia pO2≤60mmHg or oxygen saturation ≤90% in room air • Respiratory rate ≥20 breaths/min
Ventilator-acquired pneumonia (15,16)	• Onset >48 hours from start of mechanical ventilation • New or evolving infiltrate on chest X-ray	• Purulent tracheobronhchial secretions • Auscultatory findings consistent with pulmonary consolidation	• PCT ≥0.25 ng/ml • Clinical pulmonary infection score (CPIS)* ≥6
Acute pyelonephritis (17,18)	• ≥10 leukocytes/high power field in urine sediment	• Fever (tympanic or oral temperature≥38^{0}C,rectal ≥38,3^o C) • Dysuria, increased urinary frequency or urgency • Flank pain or lumbar pain at palpation • Consistent ultrasound findings	Positive leukocyte esterase in urine or WBC≥10/high power field in urine sediment

*CPIS is a clinical score based on the following parameters: body temperature, leukocyte count, volume and character of tracheal secretions, arterial oxygenation, chest radiograph findings, Gram stain results, and results of culture of tracheal aspirate specimens to predict the presence of VAP(19). Each parameter takes a score from 0 to 2 (see Appendix I).

- Patients infected with *Mycobacterium tuberculosis*.
- Patients suffering from cystic fibrosis
- Severely immunocompromised patients such as a) patients with infection by the human immunodeficiency virus and with a CD4 count of less than 200 cells/mm^3; b) neutropenic patients with less than 500 neutrophils/mm^3; and c) patients with solid organ transplantation.

Study interventions

Patients will be randomized at 1:1 ratio by a separate list per study site into two treatment groups. The list of randomization will be generated by a biostatistician and the randomization will be in a sealed envelope. The envelope will be delivered to the attending physicians after randomization. All patients will be enrolled and randomized within 24 hours from the start of antimicrobials. The two groups of treatment will be as follows:

- Standard of care: these patients will receive antimicrobials according to standard practice of the attending physicians but PCT will not be measured and antimicrobials will be stopped according to the local standard practice.
- PCT group: these patients will receive antimicrobials according to standard practice of the attending physicians and PCT will be measured on day 1 (day of start of antimicrobials) and then daily starting from day 5 (96 hours from the start of antimicrobials). Antimicrobials will be discontinued when PCT value is less than 80% of the initial value or it remains below 0.5 ng/ml. PCT will be measured by an immunoassy.

Patient follow-up and record of data

All medical and nursing charts will be reviewed by a team of physicians completely blind to theallocation group. The following information per patient will be registered into a case report form (CRF) starting from the day of startof antibiotics until discharge from hospital or death while hospitalized:

- Preexisting comorbidities
- Present case history
- Type of infection
- Vital signs

- Severity scores of SOFA, APACHE II (see Appendices II and III) and qSOFA
- Type, dose and duration of administered antimicrobial therapy
- Daily red, white and platelet cell count (if available)
- pH, pO_2, pCO_2, HCO_3 and lactate (if available)
- Glucose, urea, creatinine (if available)
- Liver biochemistry and INR (if available)
- Microbiology
- Absolute quantities of X-rays, CT- tomographies, interventions (i.e., catheterizations, tracheostomies and hemodialysis), laboratory tests (including blood cell counting, biochemistry, blood gas, and microbiology), antimicrobials, antifungals, and all other non-antimicrobial drugs
- Final disposition (dead or alive)
- Discharge from hospital

All patients discharged from hospital will be monthly followed up for their clinical status and health per phone calls until month 6. Stool specimens will be collected as described in the next section.

Collection of blood and stool

Stool specimens will be collected from all patients on days 1, 7 and 28 and in 6 months. The samples will be transferred within one hour at the central lab. The central lab is the Laboratory of Immunology of Infections of the 4th Department of Internal Medicine at ATTIKON University hospital. At the central lab upon sample arrival,

- Part of sample will be stored at -80^{0}C until microbiome analysis
- Stool sample will be cultured on three different McConkey agar plates with incorporated 16μg/ml of cefotaxime, 8μg/ml of imipenem and 250μg/ml of amikacin respectively. Each bacterial strain growing on the above plates will be identified and tested for susceptibilities by BD Phoenix Automated Microbiology System. Growth of colonies of the same strain on at least two of these plates will be considered as an index of colonization by MDR bacteria.

- All stool samples will be tested for glutamate dehydrogenase (GDH) antigenand toxins A and B of *C.difficile*by an enzyme immunoassay (EIA).

In case of diarrhea occurring between the above days of sampling, one stool sample will be transferred within the first 30 minutes to the local microbiology lab and tested by EIA for glutamate dehydrogenase (GDH) antigen and toxins A and B of Clostridium difficile (20). The gut microbiome will be evaluated from the specimens of day 1 and 28 as well as in 6 months, using 16S rRNA gene and shotgun metagenomic sequencing (21).

Costestimation

The hospitalization cost per day will be estimated by the sum of multiplications of each counted item withits price in Euros and the addition of the nominal cost of daily stay for theICU or general ward. The unit price for each counted item will derive from the official pricelist as defined by the Greek government. Counting of the items will be performed by three investigators, also completely blind to the allocated treatment. The cost of human resources (e.g., salaries of nursing and medical personnel) will not be counted.

Study endpoints

Primary endpoint

The primary study endpoint will be the change of infection-associated adverse events rate for patients treated by the PCT-guided stopping rule compared to patients treated by standard of care. The infection-associated adverse events rate is considered as the advent of any case of CDI or infection by MDR or new colonization by MDR and/or *C.difficile*or infection-associated death with the first six months from study inclusion.

For this purpose, CDI is defined asone of the following two situations (20, 22, 23):

- the presence of a positive EIA for glutamate dehydrogenase (GDH) antigen and toxins A and B of *Clostridium difficile* in a patient with a)three or more unformed stools per day, or b)toxic megacolon
- Endoscopic findings of pseudomembranes

MDRinfections are defined as any infection requiring medical treatment where there is confirmed microbiological etiology for an MDR pathogen. For this purpose the following pathogens will be considered as MDR:

- Methicillin-resistant *Staphylococcus aureus*
- Methicillin-resistant species of coagulase-negative *Staphylococcus*
- Vancomycin-resistant species of *Enterococcus*
- Gram-negative bacteria resistant to at least three of the following antibiotic classes: beta-lactamase inhibitors, antipseudomonal cephalosporins/penicillins, carbapenems, fluoroquinolones, aminoglycosides, polymyxins (24,25).

New colonization by MDR is defined as absence in stool samples on day 1 and presence in stool samples on day 7 or day 28 of growth of colonies of the same strain on at least two agar plates incorporated with antibiotics, as described above in paragraph “Collection of blood and stool”.

New colonization by C.difficile is defined as absence in stool samples on day 1 and presence in stool samples on day 7 or day 28 of a positive EIA for glutamate dehydrogenase (GDH) antigen but negative toxins A and B of *Clostridium difficile* in a patient without any clinical symptoms.

Infection-associated death is any death due to CDI or infection by MDR.

The primary endpoint is analyzed in month 6. For patients with more than one infection-associated adverse event the first one is encountered.

Secondary endpoints

The secondary endpoints are the difference between the two groups of treatment on in each of the following:

- Time to first infection-associated adverse events rate until month 6. For patients with more than one infection-associated adverse event the first one is encountered.

- Rate of CDI until month 6. For patients with more than one episode of CDI the first one is encountered.
- Rate of infections by MDR until month 6. For patients with more than one infection by MDR event the first one is encountered.
- All-cause28-day mortality
- All-cause6-month mortality
- Rate stool positive for GDH by *C.difficile* until month 6
- Rate of stool colonization by MDR until month 6
- Microbiome composition on day 28
- Changes of the microbiome between days 1 and 28and between day 1 and 6 months
- Consumption of antimicrobials until hospital discharge
- Real cost until hospital discharge.

STUDY POWER

The study is powered for the primary endpoint. It is anticipated that the rate of infection-associated adverse events in the standard-of-care group will be 30% and that by using the PCT-guided stop of antimicrobial it will be decreased to 15%. To achieve 80% power at the 5% significance level to demonstrate this difference, 133 patients should be enrolled in each arm as analyzed by the Power and Sample Size Calculation Program (version 3.1.2).To adjust for missing values, it is anticipated that 140 patients should be enrolled in each arm.

STATISTICAL ANALYSIS

Infection-associated adverse events rates and mortality rates between the two groups will be compared by the Fisher exact test. The time to an event will be compare between the two groups by the log rank test. Cost comparisons will be done as already published by our group (26). Any value of p below 0.05 will be considered as significant.

ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient's evaluation. Investigators should monitor subjects for adverse events and are responsible for recording ALL adverse events and serious adverse events occurring to a patient during the trial. Infection-associated adverse events will not be reported as AEs and SAEs since they are the study endpoints.

An adverse event is any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The time relationship is defined from the moment the AE occurs during therapeutic treatment until 30 days or 5 half-lives after treatment discontinuation. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

Serious adverse events (SAEs) must be reported to within 24 hours. If an adverse event meets any of the following criteria, it is considered 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 were more severe or were to progress.
- **Inpatient 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 a serious adverse event/experience 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 anomaly/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 any untoward medical occurrence in a patient or subject who is administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment. A non-serious adverse event is one that does not meet the definition of a serious adverse event given.

Grading of severity

The severity of the adverse events shall be graded as:

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

Relationship to the drug

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related**: The adverse event has a strong time relationship to 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 aetiology 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 aetiology.
- **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 aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate 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 Case Report Form.

STUDY TIMETABLE

- The study protocol requires three months from submission for approval by the Local Ethics Committees, by National Ethics Committee and by the National organization for Medicines.
- The study will run in three departments of Internal Medicine and in one Intensive Care unit. The study enrolment period will be 12 months.
- The clearance of the database will end three months after enrolment of the last patient.
- The statistical analysis will run for two months.

The total time period from initial submission from approval until the deliverable of the clinical primary and secondary endpoints will be 20 months.

STUDY BUDGET

This is itemized as follows:

Administration costs	
• Insurance fee	€10,000
• Submission for Local Ethics approvals	4 x €807,50= €3,230
• Submission to the National Organization for Medicines	€1,500
Personnel	
• Fee per study sire per enrolled patient	€200 x 240= €48,000
• One technician for 12 months	€500 x 12= €6,000
Consumables	
• Procalcitonin kits	To be provided by BRAHMS
• EIA for *C.difficile*	€9,600
• Reagents for stool culture	€6,000
• Cost of microbiome running and analysis	€72,000
Final cost	**€156,330**

REFERENCES

1. Paul M. Is there a treatment for sepsis other than antibiotics? ClinMicrobiol Infect2009;15:297
2. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2012. Intensive Care Med2013;39: 165-228
3. Giamarellos-Bourboulis EJ, Tsangaris I, Kanni T, et al. Procalcitonin as an early indicator of outcome in sepsis: a prospective observational study.J Hosp Infect2011;77:58-63
4. Georgopoulou A, Savva A, Giamarellos-Bourboulis EJ, et al.Early changes of procalcitonin may advise about prognosis and appropriateness of antimicrobial therapy in sepsis.J Crit Care2011;26: 331
5. Hochreiter M, Köhler T, Schweiger A, et al. Procalcitonin to guide duration of antibiotic therapy in intensive care patients: a randomized prospective controlled trial. Crit Care2009;13:83
6. Bouadma L, Luyt CE, Tubach F, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentrerandomised controlled trial. Lancet2010;375:463-474
7. de Jong E, van Oers JA, Beishuizen A,et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial.Lancet Infect Dis2016;16:819-827
8. Brown K, Valenta K, Fisman D, et al. Hospital ward antibiotic prescribing and the risks of Clostridium difficile infection. JAMA Intern Med 2015;175:626-633
9. Feazel LM, Malhotra A, Perencevich EN, et al. Effect of antibiotic stewardship programmes on Clostridium difficile incidence: a systematic review and meta-analysis. J Antimicrob Chemother2014;69:1748-1754
10. Van Schaik W. The human gut resistome. Philos Trans R SocLond B Biol2015; 370:20140087
11. Blaser MJ. Antibiotic use and its consequences for the normal microbiome. Science2016;352:544-545
12. Sommer MO, Dantas G. Antibiotics and the resistant microbiome. Current OpinMicrobiol2011;14:556-63

13. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia.ClinMicrobiol Rev1997;10:444-465
14. Müller B, Harbarth S, StolzD,et al.Diagnostic and prognostic accuracy of clinical and laboratory parameters in community-acquired pneumonia. BMC Infect Dis2007; 7: 10
15. Kalil A, Metersky M, Klompas M. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 2016;63:1-51
16. Zilberberg M, Shorr A. Ventilator-Associated Pneumonia: The Clinical Pulmonary Infection Score as a Surrogate for Diagnostics and Outcome. Clin Infect Dis 2010;51:131-135
17. Mitterberger M, Pinggera GM, Colleselli D,et al. Acute pyelonephritis: comparison of diagnosis with computed tomography and contrast-enhanced ultrasonography. BJU Int2008;101:341-344
18. Pinson AG, Philbrick JT, Lindbeck GH,et al. Fever in the clinical diagnosis of acute pyelonephritis. Am J Emerg Med1997;15:148-151
19. Zilberberg M, Shorr A. Ventilator-Associated Pneumonia: The Clinical Pulmonary Infection Score as a Surrogate for Diagnostics and Outcome. Clin Infect Dis 2010;51: 131-135
20. DuPont H, Diagnosis and management of *Clostridium difficile* infection.ClinGastroenterolHepatol2016;11:1216-1223
21. Jovel J, Patterson J, Wang W, et al. Characterization of the gut microbiome using 16S or shotgun metagenomics. Front Microbiol2016;7:459
22. Crobach MJR, Planche T, Eckert C, et al. European Society for Clinicla Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. ClinMicrobiol Infect 2006; 22: S63-S81
23. Cohen S, Gerding DN, Johnson S.Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control HospEpidemiol2010;31:431-455

24. Seligman R, Ramos-Lima LF, Oliveira Vdo A, et al. Risk factors for infection with multidrug-resistant bacteria in non-ventilated patients with hospital-acquired pneumonia. J Bras Pneumol2013;39:339-48
25. Magiorakos AP, Srinivasan A, Carey RB, et al.Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.ClinMicrobiol Infect2012;18:268-281
26. Tsaganos T, Raftogiannis M, Pratikaki M, et al. Clarithromycin leads to long-term survival and cost benefit in ventilator-associated pneumonia and sepsis. Antimicrob Agents Chemother 2016;60:3640-3646

APPENDIX I The clinical pulmonary infection score

CPIS points	**0**	**1**	**2**
Tracheal secretions	Rare	Abundant	Purulent
Leukocyte count (/mm³)	>4.000 and <11.000	<4.000 and >11.000	<4.000 or >11.000+band forms
Temperature (ºC)	>36.5 and <38.4	>38.5 and <38.9	>39 or <36
pO_2/FiO_2 ratio (mmHg)	>240 or ARDS	-	≤240 and no ARDS
Chest radiograph	No infiltrate	Diffuse infiltrate	Localised infiltrate
Culture of tracheal aspirate	Negative	-	Positive

APPENDIX II TheAPACHE II score

PARAMETER	VALUES ABOVE NORMAL				NORMAL	VALUES BELOW NORMAL			
	+4	+3	+2	+1	0	+1	+2	+3	+4
1. Rectal temperature (^{o}C)	>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: onlyPaO_2					PaO_2>70	PaO_2 61-70		PaO_2 55-60	PaO_2<55
6. ArterialpH	>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 solium (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		20-39.9	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) To be used only if absent arterial gas	52	41-51,9		32-40,9	22-31,9		18-21,9	15-17,9	

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 he is immunodeficient scoring is done as follows::

α. No surgery or emergency surgery: + 5 points

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

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

APPENDIX III The SOFA score

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<70mmHg	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