INTEGRATION OF CLINICAL AND LABORATORY INFORMATION TO GENERATE TECHNOLOGICAL ADVANCE FOR THE DIAGNOSIS OF SEPSIS: THE INTELLINGENCE STUDY

CLINICAL STUDY PROTOCOL

Study acronym: INTELLIGENCE/HemoSpec

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DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: INTEGRATION OF CLINICAL AND LABORATORY INFORMATION TO GENERATE TECHNOLOGICAL ADVANCE FOR THE DIAGNOSIS OF SEPSIS: THE INTELLINGENCE STUDY

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 I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.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

STUDY SYNOPSIS

Aim	A diagnostic devise, namely HemoSpec, had been developed that integrates clinical information, along with information on circulating protein biomarkers and the morphology of white blood cells to achieve early diagnosis of sepsis. The current study is aiming to validate and improve performance of HemoSpec for the rapid assessment of the critically ill patient.
Design	Prospective phase II study
Inclusion criteria	1. Age above or equal to 18 years old 2. Both genders 3. Written consent provided from patients or their first-degree relatives for patients unable to consent 4. Presence of at least two signs of the systemic inflammatory response syndrome (SIRS) with the onset of the first sign the last 24 hours 5. SIRS due to multiple injuries, acute pancreatitis, post-operation or due to clinical signs of infection.
Study groups	• Patients with sepsis defined by the Sepsis-3 definition • Patients without sepsis; this group comprises both patients with infection and patients with SIRS without infection
Primary study endpoint	The sensitivity of HemoSpec output to diagnose the presence of sepsis compared to the absence of sepsis. HemoSpec will be considered to provide a satisfactory diagnosis of sepsis is sensitivity for the diagnosis is greater than 85%.
Secondary study endpoints	• The specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to diagnose the presence of sepsis compared to the absence of sepsis. • The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict unfavorable outcome after 28 days compared to survivors • The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict unfavorable outcome after 90 days compared to survivors • The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict development of organ dysfunction during the 28 days of follow-up.

	• The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to indicate patients with infection among those scoring positive for qSOFA • The comparison of the diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output to diagnose the presence of sepsis between patients with microbiologically-proven infection and patients without microbiologically-proven infection • Difference of the diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output between patients with sepsis and septic shock compared to patients without sepsis • The diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output for the progression of a patient into organ failure during the first 28-days
Exploratory endpoint	Financial savings with the introduction of HemoSpec as a diagnostic device for sepsis. This will be analyzed building a model and feeding the model using financial data coming from recent publications in Greece
Power of the study	The power of the study is defined by the study primary endpoint. It is anticipated that to disclose 85% sensitivity of HemoSpec output for the diagnosis of sepsis with power of 90% at the level of 5%, 220 patients should be enrolled in total.

LIST OF ABBREVATIONS

ABTI: acute biliary tract infection

AIS: abbreviated injury scale

AP: acute pyelonephritis

APACHE: acute physiology and chronic health evaluation

ARDS: acute respiratory distress syndrome

ASP: acute secondary peritonitis

BSI: bloodstream infections

CAP: community-acquired pneumonia

CCI: Charlson's Comorbidity Index

CI: confidence interval

CPIS: clinical pulmonary infection score

CRF: case report form

GCS: Glasgow Coma Scale

HAP: hospital-acquired pneumonia

HCAP: health-care associated pneumonia

IAA: intraabdominal abscess

ICU: Intensive Care Unit

INR: international normalized ratio

ISS: injury severity score

MAP: mean arterial pressure

MODS: multiple organ dysfunction syndrome

NPV: negative predictive value

PCT: procalcitonin

PO_2/FiO_2: ratio of partial oxygen pressure to fraction of inspired oxygen

PPV: positive predictive value

qSOFA: quick SOFA score

SIRS: systemic inflammatory response syndrome

SOFA: sequential organ failure assessment

suPAR: soluble urokinase plasminogen activator receptor

VAP: ventilator-associated pneumonia

INTRODUCTION

Sepsis is a life-threating organ dysfunction resulting from the dysregulated response of the host to an infection[1]. It is estimated that 1.5 million people present with sepsis annually in Northern America and another 1.5 million people in Europe; 30 to 50% of them die making sepsis the leading cause of death[2]. The key-point in the management of sepsis is the early resuscitation with broad-spectrum antimicrobials and intravenous fluids, if possible within the first hour[3]. The great mortality of sepsis indicates that this goal is not easy to be achieved for two main reasons: the first is the delay in recognition of the septic patients and the second is the resistance of the implicated pathogen to broad-spectrum antimicrobials[4].

In an attempt to improve the failure of physicians for early sepsis recognition, several markers have been developed. Some of them rely on clinical signs of the host and others on the measurements of circulating biomarkers. Recently, qSOFA (quick SOFA score) has been introduced to help the early recognition of sepsis in patients who present with infection outside the Intensive care Unit (ICU) i.e. either in the community or during hospitalization in the general ward[1]. However, there are concerns of the sensitivity of qSOFA and many introduce the need to measure biomarkers in serum. These biomarkers are usually protein molecules that are over-produced in the host as a result of the interaction with an infective insult. However, these protein molecules are produced by white blood cells. What is currently known is that although most of patients present with a similar phenotype, their pathophysiology is diverse. More precisely, although the majority of patients with sepsis present with high concentrations of protein molecules like interleukin (IL)-6, C-reactive protein (CRP) and procalcitonin (PCT) in their blood[5-7], in some patients circulating white blood cells remain over-active and in other patients they are significantly anergic, a situation often known as sepsis-induced immunoparalysis[8]. Another molecule, called soluble urokinase plasminogen activator receptor (suPAR), is the shed uPAR receptor on neutrophils and is released in the circulation as a result of neutrophil activation[9]; concentrations greater than 12 ng/ml can trace with negative predictive value almost 95% the patient at great chance of unfavorable outcome. As such, the robust diagnosis of sepsis may rely on a combination of clinical assessment, measurement of protein biomarkers and validation of the activity of circulating white blood cells.

One FrameWork 7-funded initiative from seven European countries aims to develop a rapid score that can integrate all clinical and laboratory information and provide early diagnosis whether a patient has sepsis or not. The vision of this initiative is to build a device that is called HemoSpec. With this approach, whole blood coming from patients will be in parralel analyzed into three aspects: a) absolute white blood cell counting; b) information on the fluidity and activity of the white blood cells using Raman spectroscopy; and c) measurement of serum levels of IL-6, CRP, PCT and suPAR. The end result is building a diagnostic algorithm where clinical information is also taken into consideration.

The project was started in November 2013 and the HemoSpec device is anticipated to be ready by November 2016. The diagnostic performance of HemoSpec is currently based on preliminary data coming from 60 patients (20 controls, 20 with systemic inflammatory response syndrome and 20 with sepsis) hospitalized in Jena University Hospital. The current study is aiming to validate and improve performance of HemoSpec for the rapid assessment of the critically ill patient in a larger phase II diagnostic study.

STUDY DESIGN

1.Patient population: Inclusion and exclusion criteria

This a prospective, phase II study that will be conducted after approval by the Ethics Committees of the Hospitals of the participating study sites. Written consent will be provided from patients or their first-degree relatives for patients unable to consent. The study will be conducted for a total period of 18 months. The study will be registered at ClinicalTails.gov.

Enrolled patients should meet ALL the following inclusion criteria:

- Age above or equal to 18 years old
- Both genders
- Written consent provided from patients or their first-degree relatives for patients unable to consent
- Presence of at least two signs of the systemic inflammatory response syndrome (SIRS) with the onset of the first sign the last 24 hours
- SIRS due to multiple injuries, acute pancreatitis, post-operation or due to clinical signs of infection.

Patients who meet ANY of the below criteria CANNOT be enrolled:

- Known infection by the human immunodeficiency virus-1;
- Neutropenia defined as an absolute neutrophil count lower than 1000 neutrophils/mm^3 due to reasons other than SIRS.

Definitions related to the inclusion criteria

1.1 Signs of SIRS (systemic inflammatory syndrome)[11]

- Temperature> 38^0C or <36^0C
- Heart rate> 90 beats/min
- Respiratory rate> 20 breaths/min or Pco_2 <32mmHg
- Leukocytosis (white blood cells > 12.000/mm^3) or leukopenia (white blood cells <4.000/mm^3) or> 10% blasts in peripheral blood

1.2 Multiple injuries are defined by the presence of **ALL** the following[12]:

- Injury severity score (ISS) more than or equal to 15. For the purposes of the study ISS is calculated based on the formula provided in APPENDIX I; and
- Absence of infection as defined by negative findings on physical examination and sterile cultures

1.3 Acute pancreatitis is defined by the presence of[13]:

- Typical epigastric pain radiating in the back

plus ONE of the following:

- Serum amylase at least three time above the upper normal limit or
- Urine amylase at least three time above the upper normal limit or
- Radiographic signs in abdominal ultrasound compatible with acute pancreatitis or
- Radiographic signs in computed tomography of the upper abdomen compatible with acute pancreatitis

1.4 Post-operation: refers to patients with ALL the following:

- Undergoing either elective colectomy or elective replacement of abdominal aorta aneurysm or coronary-arterial by-pass surgery
- The first sign of SIRS develops less than 24 hours after operation

1.5 Clinical infection

Patients may have one of the following well-characterized infections: community-acquired pneumonia, health-care associated pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia, acute pyelonephritis, acute biliary tract infection, intrabdominal abscess, acute secondary peritonitis or primary bloodstream infection. Each infection is defined by the following criteria:

1.5.1 Community-acquired pneumonia (CAP)[14] as a patient without any history of contact with the hospital environment or with health-care facilities the last 90 days and presenting at least two of the following clinical/laboratory signs: a) dyspnea; b) purulent expectoration; c) auscultatory rales; and d) new consolidation in chest X-ray.

1.5.2 Health-care associated pneumonia (HCAP)[15] as a patient either residing in a long-term care facility or undergoing chronic hemodialysis or hospitalized the last 30 days and who presents at least two of the following clinical/laboratory signs: a) dyspnea; b) purulent expectoration; c) auscultatory rales; and d) new consolidation in chest X-ray.

1.5.3 Hospital-acquired pneumonia (HAP)[15] as a patient who presents at least two of the following clinical/laboratory signs: a) dyspnea; b) purulent expectoration; c) auscultatory rales; and d) new consolidation in chest X-ray. These signs should present at least 48 hours after hospital admission and infection should not be under development during hospital admission.

1.5.4 Ventilator-associated pneumonia (VAP)[16] as a patient who meets ALL the following criteria:

- Intubation and mechanical ventilation for at least 48 hours;
- Purulent tracheobronchial secretions (TBS);
- New consolidation in chest X-ray or progression of a former infiltrate in chest X-ray; and
- Clinical pulmonary infection (CPIS) score more than 6; CPIS is calculated using the formula provided in APPENDIX II.

1.5.5 Acute pyelonephritis (AP)[17] is defined by the presence of at least one clinical signs and one laboratory finding. Clinical signs are:

- Dysuria or frequency in urination;
- Flank pain;
- Pain induced after deep palpation of the right or left costo-vertebral angle

Laboratory findings are:

- Pyuria defined as more than 10 white blood cells per high power field of spun urine;
- Ultrasound findings compatible with acute pyelonephritis
- Radiological findings from computed urography compatible with acute pyelonephritis

1.5.6 Acute biliary tract infection (ABTI)[18] Every patient who meets at least two of the following signs:

- Nausea and vomiting starting the last 24 hours
- Pain at the upper right quadrant starting the last 24 hours
- Jaundice starting the last 24 hours
- Pain at deep palpation of the upper right quadrant

AND

who has radiological findings on abdominal ultrasound or computed tomography of the upper abdomen compatible with acute biliary infection. These radiological findings may be one of the following: dilatation of the gallbladder, increased thickness of the wall of the gallbladder, exudation of the wall of the gallbladder, dilatation of the extrahepatic common bile duct or dilatation of the intrahepatic biliary tracts

1.5.7 Intrabdominal abscess (IAA)[18] Every patient who presents with radiological findings on abdominal ultrasound or computed tomography of the abdomen typical of an intraabdominal abscess. These characteristics should be the well-definition of the dimensions of the abscess and intraabdominal location. The abscess may be localized either inside the liver parenchyma or in the kidney or within the intestines or in the pelvis.

1.5.8 Acute secondary peritonitis (ASP)[18] Every patient who meets at least TWO of clinical signs and at least ONE the evidence signs. The clinical signs are:

- Decreased bowel sounds
- Start of abdominal pain at the last 24 hours
- Rebound abdominal tenderness

The evidence signs are:

- Radiological findings on abdominal X-ray or computed tomography of the abdomen compatible with acute secondary peritonitis showing free air in the abdominal cavity resulting from the rupture of an organ
- Findings compatible with ASP in open abdominal surgery

1.5.9 Primary bloodstream infection (BSI)[18] Every patient who meets ALL the following signs:

- Peripheral blood culture positive for Gram-positive or Gram-negative bacteria or fungal species. Coagulase-negative *Staphylococcus* spp and skin commensals are considered contaminants unless isolated at least two times or isolated from both a peripheral vein and a central catheter and they have the same antibiogram
- Absence of any primary site of infection after an extensive patient work-out.

2. Interventions

For every patient 10 ml of blood are sampled after venipuncture of one forearm vein under aseptic conditions. From this volume, 3 ml is collected into one EDTA-coated tube, 3 ml is collected into one heparin-coated tube and 4 ml are collected into one sterile and pyrogen-free tube. No other intervention will be done to the enrolled patients. Day of sampling is defined as day 1.

3. Follow-up

For every patient The following information is recorded:

- Complete case history. Major emphasis is given on the collection of information regarding comorbidities and predisposing conditions. Recorded comorbidities comprise but are not limited to: diabetes mellitus type 1 or type 2; chronic heart

failure; chronic obstructive or restrictive pulmonary disorder; rheumatic diseases; chronic intake of corticosteroids or of disease-modifying agents; chronic renal disease; solid tumor malignancy; hematological malignancies and intake of chemotherapy. Disease modifying agents comprise any anti-cytokine biological agent and any chemical non-biological agent (e.g. cyclophosphamide). Predisposing conditions comprise but are not limited to: stroke, brain hemorrhage, dementia, degenerative brain disease, myocardial infarction, gallstones, nephrolithiasis and any other surgery. For patients who are enrolled for infections presenting at least 48 hours after ICU admission, the baseline SOFA score on ICU admission shall be recorded. For all patients Charlson's Comorbidity Index (CCI) will be calculated[19]. CCI is provided in APPENDIX III.

- Specific recording on day 1 before blood sampling of the three elements of the qSOFA score for patients presenting with infections outside the ICU[1]: a) more than 22 breaths/minute; b) sudden alteration of mental status; and c) systolic arterial pressure less than 100mmHg.
- Complete diagnostic work-out on day 1 comprising a) blood cell counting; b) biochemistry; c) blood culture and culture of other biological fluids if possible like urine culture and quantitative TBS cultures; d) chest X-ray and renal or abdominal ultrasound or computed tomography if considered necessary; e) APACHE II score. Acute physiology and chronic health evaluation (APACHE) II score is calculated based on the information provided in APPENDIX IV.
- Everyday recording from day 1 until day 28 of the following (if available by the patient chart): a) need for blood cultures; b) results of microbiology and antibiogram of the isolated microorganism(s); c) SOFA score; d) administered antimicrobials; e) administered non-antimicrobial drugs; f) failing organs; g) the ratio of oxygen partial pressure to fraction of inspired oxygen; h) the most deteriorated vital signs of the day; and i) Glasgow coma scale. Sequential organ failure assessment (SOFA) score is calculated based on the information provided in APPENDIX V[1].
- Outcome on day 28 (death or survival) with recording of the exact day of death in case of death
- Outcome on day 90 (death or survival) with recording of the exact day of death in case of death

3.1 Definitions of organ failures[11]

- Acute respiratory failure as the presence of a) diffuse bilateral shadows in chest X-ray and b) ratio of oxygen partial pressure to fraction of inspired oxygen below 200.
- Acute renal dysfunction as urinary output below 0.5 ml//Kg BW/h for at least two consecutive hours provided that the patient's negative fluid balance has been restored
- Acute coagulopathy as platelet count <100.000/mm^3 or INR (international normalized ratio> 1.5
- Cardiovascular failure as any mean arterial pressure below 65mmHg not responding to fluid resuscitation and accompanied by lactate more than 2 mmol/l and requiring the administration of vasopressors

Multiple organ dysfunction syndrome (MODS) is defined as the disruption of two or more systems (respiratory, renal, metabolic acidosis, coagulation disorders, cardiovascular), which require exogenous intervention (mechanical ventilation, haemofiltration, transfusions, inotropic drugs) to maintain homeostasis.

4.HemoSpec analysis

All blood samples are transported to the Research Department of Immunology of Infectious Diseases of the Research Laboratory of Infectious Diseases and Antimicrobial Therapy of the 4th Department of Internal Medicine at the Medical School of University of Athens. The samples will be analyzed using the HemoSpec device installed there. The output of HemoSpec will provide the possibility that a patient has sepsis.

4.Monitoring

All clinical and laboratory information are recorded in a Case Report Form (CRF). All CRFs are monitored by two independent monitors: one monitor blind to the laboratory information and who monitors the correct recording of clinical data; and one monitor blind to the clinical information who monitors the correct recording of laboratory data.

STUDY ENDPOINTS

Primary endpoint

- The sensitivity of HemoSpec output to diagnose the presence of sepsis compared to the absence of sepsis. HemoSpec output will be considered to provide a satisfactory diagnosis of sepsis if sensitivity for the diagnosis is greater than 85%.

Secondary endpoints

- The specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to diagnose the presence of sepsis compared to the absence of sepsis.
- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict unfavorable outcome after 28 days compared to survivors
- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict unfavorable outcome after 90 days compared to survivors
- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to predict development of organ dysfunction during the 28 days of follow-up.
- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpec output to indicate patients with infection among those scoring positive for qSOFA
- The comparison of the diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output to diagnose the presence of sepsis between patients with microbiologically-proven infection and patients without microbiologically-proven infection
- Difference of the diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output between patients with sepsis and septic shock compared to patients without sepsis.
- The diagnostic performance (sensitivity, specificity, PPV and NPV) of HemoSpec output for the progression of a patient into organ failure during the first 28-days

Exploratory endpoint

- Financial savings with the introduction of HemoSpec as a diagnostic device for sepsis. This will be analyzed using financial data coming from recent publications in Greece[20,21] and building a model

Definitions for study endpoints

Sepsis This is defined separately for patients presenting with infections outside the ICU and for patients presenting with infections after ICU admission.

For patients outside the ICU, sepsis is defined as the presence of one microbiologically confirmed or clinically diagnosed infection accompanied by SOFA score greater than or equal to 2 according to the Sepsis-3 definitions[1].

For patients inside the ICU, sepsis is defined as the presence of one microbiologically confirmed or clinically diagnosed infection accompanied by an increase of baseline SOFA score greater than or equal to 2 according to the Sepsis-3 definitions[1].

Septic shock is defined as the presence of ALL the following[1]:

- MAP below 65 mmHg not responding to fluid resuscitation
- Blood lactate equal to or greater than 2 mmol/l
- Need for vasopressors

Microbiological confirmation: Isolation of a microorganism considered by the attending physicians to be the pathogen in at least one body fluid culture.

Development of organ failure Organ failures are defined as above in the protocol (page 14, section 3.1)

POWER CALULATION

The study is powered for the primary endpoint. The formula used to calculate the needed number of enrolled patients is:

$$N= z^2p(1-p)/e^2$$

where N is the sample size required (provided the study population is not limited, which is actually the case); z is the value from the standard normal distribution corresponding to desired confidence level of the estimation of the proportion. Assuming 95% CI, z=1.96; p is the expected true proportion i.e.0.85 and (1-p)=0.15; and e is the desired precision which is equal to half desired confidence interval (CI) width, hence e=10%/2=0.05. This is the allowable or acceptable error in the estimate.

Substituting the numbers in the equation we get N=199. Taking into consideration that some patients will be excluded from analysis due to incomplete dataset, we assume that a total of 220 patients need to be enrolled.

STATISTICAL ANALYSIS

For analysis purposes, patients are divided into two large groups; those with sepsis and those without sepsis as defined by the Sepsis-3 criteria. The group of patients without sepsis comprises patients with infection and patients with SIRS without infection. Sensitivity, specificity, PPV and NPV for all endpoints will be provided as percentage (%) and 95% CIs. Comparisons between patients with and without microbiological confirmation of infections will be done by the Fisher exact test.

Financial data from patients with sepsis enrolled in two prospective trails in Greece and allocated to placebo treatment are available[20,21]. From this pool of patients, patients with sepsis of similar severity and comorbidities will be selected to match to patients enrolled in the current study. A model of probable financial savings will be built testing the hypothesis that faster diagnosis with HemoSpec leads to faster management with better outcomes.

SAFETY

The study design does not raise any safety issues because the only intervention will be blood sampling on day 1.

REFERENCES

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315: 801-810
2. Stevenson EK, Rubenstein AR, Radin GT, Wiener RS, Walkey AJ. Two decades of mortality trends among patients with severe sepsis: a comparative meta-analysis. *Crit Care Med* 2014; 42: 625-631
3. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013; 41: 580-637
4. Koupetori M, Retsas T, Antonakos N, Vlachogiannis G, Perdios I, Nathanail C, et al. Bloodstream infections and sepsis in Greece: over-time change of epidemiology and impact of de-escalation on final outcome. *BMC Infect Dis* 2014; 14: 272
5. Becker KL, Snider R, Nylen ES. Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. *Crit Care Med* 2008; 36: 941-952
6. Tang BMP, Eslick GD, Craig JC, McLean AS. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. *Lancet Infect Dis* 2007; 7: 210-217
7. Dahaba AA, Hagara B, Fall A, Rehak PH, List WF, Metzler H. Procalcitonin for early prediction of survival outcome in postoperative critically ill patients with severe sepsis. *Br J Anesth* 2006; 97: 503-508
8. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis 2013; 13: 260-268.
9. Giamarellos-Bourboulis EJ, Norrby-Teglund A, Mylona V, Savva A, Tsangaris I, Dimopoulou I, et al. Risk assessment in sepsis: a new prognostication rule by APACHE II score and serum soluble urokinase plasminogen activator receptor. *Crit Care* 2012; 16: R149
10. Advanced spectroscopic hemogram for personalized care against live threatening infections using an integrated chip-assisted bio-photonic system, 7th Framework Program project 611682

11. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1995; 20: 864-874
12. Paraschos MD, Patrani M, Pisitki A, Katsenos C, Tsaganos T, Netea MG, et al. Defective cytokine production early after multiple traumas: modulation in severe sepsis. *Cytokine* 2015; 76: 222-226
13. Mylona V, Koussoulas V, Tzivras D, Makrygiannis E, Georgopoulou P, Koratzanis G, et al. Changes in adaptive and innate immunity in patients with acute pancreatitis and systemic inflammatory response syndrome. *Pancreatology* 2011; 11: 475-481
14. Christ-Crain M, Stolz D, Bingisser R, Müller C, Miedinger D, Huber PR, et al. Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia. A randomized trial. *Am J Resp Crit Care Med* 2006; 174: 84-93
15. Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2002, 165: 867-903
16. Rello J, Paiva JA, Baraibar J, Barcenilla F, Bodi M, Castander D, et al. International conference for the development of consensus on the diagnosis and treatment of ventilator-associated pneumonia. *Chest* 2001, 120: 955-970
17. Pinson AG, Philbrick JT, Lindbeck GH, Schorling JB. Fever in the clinical diagnosis of acute pyelonephritis. *Am J Emerg Med* 1997; 15: 148-151
18. Calandra T, Cohen J. The International Sepsis Forum Consensus definitions of infections in the intensive care unit. *Crit Care Med* 2005; 33: 1639-1648
19. Charslon ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373-383
20. Giamarellos-Bourboulis EJ, Mylona V, Antonopoulou A, Tsangaris I, Koutelidakis I, Marioli A, et al. Effect of clarithromycin in patients with suspected Gram-negative sepsis: results of a randomized controlled trial. *J Antimicrob Chemother* 2014; 69: 1111-1118
21. Tsaganos T, Raftogiannis M, Pratikaki M, Christodoulou S, Kotanidou A, Papadomichelakis E, 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: Calculation of the Injury Severity Score (ISS)

For the calculation of ISS injury of each of the head, thorax, abdomen, pelvis and extremities is scored from 1 to 6 based on the Table of AIS provided below. Then the sum of the scores at the power of 2 of three most severely scored regions is calculated. ISS can get values from 0 to 75. Once a region is scored by AIS as 6, then the total ISS is automatically 75 irrespective of the presence of other injuries or not.

Abbreviated Injury Scale (AIS)	Characterization of the injury
1	Small
2	Moderate
3	Severe
4	Very severe
5	Critical
6	Non-viable

APPENDIX II: Clinical pulmonary infection score (CPIS)

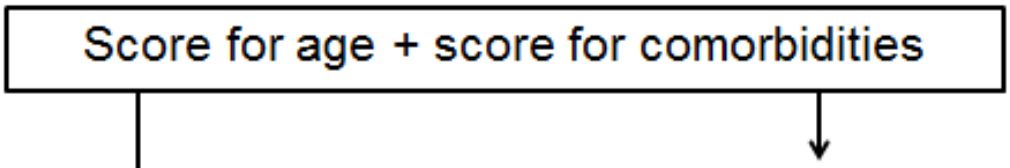
CPIS is caclulated by the sum of scoring for each parameter provided in the Table below.

Score	**0**	**1**	**2**
Tracheobronchial secretions	None	Aberrant no purulence	Aberrant and purulent
Inflitrate in chest X-ray	None	diffuse	Localized
Temperature (^{0}C)	36.5-38.4	38.5-38.9	<36.0 or >39.0
White blood cells (/mm^3)	4000 - 11000	<4000 or >11000	<4000 or >11000 and ≥50% bands
PaO_2/FiO_2 (mmHg)	>240 or ARDS		≤240 in absence of ARDS
Quantitative cutlures of TBS	No growth	Moderate growth	Growth >10^5 cfu/ml with positive Gram stain

ARDS: acute respiratory distress syndrome

PaO_2/FiO_2: the ratio of partial oxygen pressure to the fraction of inspired oxygen

APPENDIX III: Calculation of the Charlson's Comorbidity Index (CCI)



Age (years)	Points
0-49	0
50-59	1
60-69	2
70-79	3
80-89	4
90-99	5

Co-morbidity	Points
None	0
Myocardial infarct, congestive heart failure peripheral vascular disease, cerebrovascular disease (except hemiplegia), dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, mild liver disease, diabetes (without complications)	1
Diabetes with end organ damage, hemiplegia, moderate or severe renal disease, solid tumor (non-metastatic), leukemia/lymphoma/multiple myeloma	2
Moderate or severe liver disease	3
Metastatic solid tumor	6
AIDS (acquired immunodeficiency syndrome)	6

APPENDIX IV: Calculation of the Acute Physiology and Chronic Health Evaluation (APACHE II) score

PARAMETER	HIGH ABNORMAL VALUES				NORMAL	LOW ABNORMAL VALUES			
	+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 blood pressure (mm Hg)	>160	130-159	110-129		70-109		50-69		<49
3. Pulse rate (ventricular rythm)	>180	140-179	110-139		70-109		55-69	40-54	<39
4. Respiratory rate:	>50	35-49		25-34	12-24	10-11	6-9		<5
5. Oxygenation: $AaDO_2$ ή PaO_2 (mm Hg)									
α) FiO_2>0. 5: calculate $AaDO_2$	>500	350-499	200-349		<200				
β) FiO_2<0. 5: note 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) (double score 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. Glascow coma score	15 – GCS =								
Total Acute Physiology Score (APS)	Total score of parameters 1-12								

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

Score of Chronic Disease

If the patient has a history of organ failure or he is immunodeficient, his score is given as follows:

- No surgical patient or surgery on emergency basis: +5 points
- Patients undergoing programmed operation: +2 points

PATIENT'S TOTAL APACHE II SCORE: APS + POINTS FOR AGE + POINTS FOR CHRONIC DISEASE=----------

APPENDIX V: Calculation of the Sequential Organ Failure Assessment (SOFA) score

	0	**1**	**2**	**3**	**4**
pO_2/FiO_2	≥400	<400	<300	<200	<100
Platelets ($x10^3$ mm^3)	≥150	<150	<100	<50	<20
Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12.0
Cardiovascular	MAP ≥70mmHg	MAP <70mmHg	<5 µg/kg/min of dopamine	≤1 µg/kg/min of norepinephrine	>1 µg/kg/min of norepinephrine
Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Creatinine (mg/dl) (or urine/day)	<1.0	1.2-1.9	2.0-3.4	3.5-4.9 (<500)	≥5.0 (<200)

MAP: mean arterial pressure