INTEGRATION OF CLINICAL AND LABORATORY INFORMATION TO GENERATE TECHNOLOGICAL ADVANCEFOR THE DIAGNOSIS OF SEPSIS: THE INTELLINGENCE-2 STUDY

CLINICAL STUDY PROTOCOL

Study acronym: INTELLIGENCE-2

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Protocol Version: 1

Date: 12 December 2016

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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-2 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 I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.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 patient admitted in the emergency department.
Design	Prospective phase III study
Inclusion criteria	1. Admission at the ED 2. Age above or equal to 18 years old 3. Both genders 4. Written consent provided from patients or their first-degree relatives for patients unable to consent 5. Considerable risk of death as as indicated by the presence of at least one of the following: i) sudden alteration of mental status; ii) systolic blood pressure less than 100 mmHg; and iii) high respiratory rate defined as more than or equal to 22 breaths per minute.
Study groups	• Patients with sepsis defined by the Sepsis-3 definition • Patients without sepsis; this group comprises both patients with infection and patients without infection
Primary study endpoint	The diagnostic performance of HemoSpec output to diagnose the presence of sepsis compared to the absence of sepsis the first 96 hours. The diagnostic performance is considered to be the constellation of specificity, positive predictive value (PPV) and negative predictive value (NPV) as expressed by the Area Under Curve (AUC) of the Receiver Operator Characteristics (ROC) curve. This is desired to be 0.9 (90%).
Secondary study endpoint	The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpecoutput to predict unfavorable outcome after 28 days compared to survivors
Power of the study	Using the F statistic, to achieve the desired AUC of the ROC curve with 80% power at the 5% level of significance considering that almost 70% of patients meeting the inclusion criteria will have sepsis, it is calculated that a total of 60 patients need to be enrolled in the entire study in both study sites..

LIST OF ABBREVATIONS

ABTI: acute biliary tract infection

AP: acute pyelonephritis

APACHE: acute physiology and chronic health evaluation

AUC: area under curve

BSI: bloodstream infections

CAP: community-acquired pneumonia

CCI: Charlson's Comorbidity Index

CI: confidence interval

CRF: case report form

CRP: C-reactive protein

ED: emergency department

GCS: Glasgow Coma Scale

HCAP: health-care associated pneumonia

IAA: intraabdominal abscess

IL: interleukin

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

ROC: receiver operator characterstics

SOFA: sequential organ failure assessment

SP: acute secondary peritonitis

suPAR: soluble urokinase plasminogen activator receptor

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]. However it is not easy to achieve this goal particularly among patients admitted in the emergency department (ED) because the diagnosis of an infection often delays until the enire workout that comprises radiology and laboratory tests is complete.

In order to help the early diagnosis of sepsis for patients arriving in the ED, several biomarkers have been developed. Most broadly used biomarkers are the absolute white blood cell count, C-reactive protein (CRP) and procalcitonin (PCT). HemoSpec[4] is a device that is able to integrate information of the patient with blood analysis. Analysis is providing information on the absolute blood cell counts, the morphology of white blood cells, CRP, PCT, interleukin (IL)-6 and soluble urokinase plasminogen activator receptor (suPAR). The software of this device is complicated by all the above type of information that is coming from prospectively collected cohorts of patients from Greece and Germany. The diagnostic performance of HemoSpec has until now validated into two phase II studies; the first has been conducted in Germany and enrolled 60 patients (20 controls, 20 with systemic inflammatory response syndrome and 20 with sepsis) hospitalized in Jena University Hospital; the second is currently ongoing in Greece and aims to use information using HemoSpec for the prospective classification of patients with documented infection into those with sepsis and into those without sepsis.

The above two trials share a common phase II design i.e. to validate the diagnostic performance of HemoSpec among patients with clinical diagnosis. The shear clinical validity of HemoSpec can be proven after a multicenter prospective study among patients admitted in the ED. This study aims to evaluate the diagnostic performance of patients admitted in the ED with clinical signs bearing a certain risk for death making them likely to suffer from sepsis.

STUDY DESIGN

1.Patient population: Inclusion and exclusion criteria

This a prospective, phase III 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 6 months.The first four months the study will be conducted among patients admitted at the ED of ATTIKON University hospital of Athens. At the last two months, the study will be conducted among patients admitted at the ED of Jena University Hospital. The study will be registered at ClinicalTails.gov.

Enrolled patients should meet ALL the following inclusion criteria:

- Admission at the ED
- Age above or equal to 18 years old
- Both genders
- Written consent according to the local rules
- Considerable risk of death as as indicated by the presence of at least one of the following: i) sudden alteration of mental status; ii) systolic blood pressure less than 100 mmHg; and iii) high respiratory rate defined as more than or equal to 22 breaths per minute.

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

- Known infection by the human immunodeficiency virus-1;
- Acute myocardial infarction as documented by positive electrocardiographic findings of ST-segment elevation
- Single trauma or multiple injuries
- Pregnancy and lactation

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. The baseline SOFA score on ICU admission shall be recorded. For all patients Charlson's Comorbidity Index (CCI) will be calculated[4]. CCI is provided in APPENDIX I.
- 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 II.
- Sequential organ failure assessment (SOFA) score as calculated based on the information provided in APPENDIX III[1].
- Final diagnosis of an infection within the next 96 hours from ED admission according to the criteria set in APPENDIX III.
- Final diagnosis of sepsis within the next 96 hours from ED admission (see below)
- Outcome on day 28 (death or survival) with recording of the exact day of death in case of death

4.HemoSpec analysis

All blood samples are transported to the central lab. This will be: a) the Research Department of Immunology of Infectious Diseases of the Research Laboratory of Infectious Diseases and Antimicrobial Therapy of the 4thDepartment of Internal Medicine at the Medical School of University of Athens for the part of the study that will be .conducted at the ED of ATTIKON hospital; and b) the Laboratoyr of the Center for Sepsis Control and Care for the part of the study that will be conducted at the ED of Jena University hospital. The samples will be analyzed using the HemoSpec device installed there. The output of HemoSpecwill 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 one monitor blind to the laboratory information.

STUDY ENDPOINTS

Primary endpoint

The diagnostic performance of HemoSpecoutput to diagnose the presence of sepsis compared to the absence of sepsis the first 96 hours. The diagnostic performance is considered to be the constellation ofspecificity, positive predictive value (PPV) and negative predictive value (NPV) as expressed by the Area Under Curve (AUC) of the Receiver Operator Characteristics (ROC) curve. This is desired to be 0.9 (90%).

Secondary endpoint

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of HemoSpecoutput to predict unfavorable outcome after 28 days compared to survivors

Definitions for study endpoints

Sepsis This is defined as the presence of one microbiologically confirmed or clinically diagnosed infection accompanied by SOFA score greater than or equal to 2[1]. For this endpoint, SOFA score is calculated according to the information provided in

APPENDIX III and the infections are diagnosed based on the criteria provided in APPENDIX IV and that are based on available publications.

POWER CALULATION

The study is powered for the primary endpoint. Using the F statistic, to achieve the desired AUC of the ROC curve with 80% power at the 5% level of significance considering that almost 65% of patients meeting the inclusion criteria will have sepsis[10], it is calculated that a total of 60 patients need to be enrolled in the entire study in both study sites.

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. Sensitivity, specificity, PPV and NPV for all endpoints will be provided as percentage (%) and 95% CIs.

SAFETY

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

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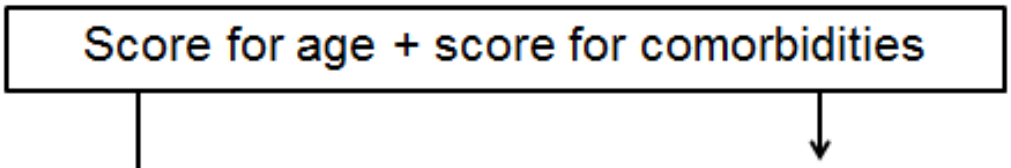
APPENDIX I: Calculation of the Charlson's Comorbidity Index (CCI)



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 II: 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 (^{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 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. 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 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 III: 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

APPENDIX IV Definitions of infections

Infection	**ALL the following**	**At least two of the following:**
Community-acquired pneumonia (CAP)[6]	No history of contact with the hospital environment or with health-care facilities the last 90 days	• Dyspnea • Purulent expectoration • Auscultatory rales • New consolidation in chest X-ray.
Health-care associated pneumonia (HCAP)[7]	Residence in a long-term care facility or chronic hemodialysis or hospitalization the last 30 days	• Dyspnea • Purulent expectoration • Auscultatory rales • New consolidation in chest X-ray.
Acute pyelonephritis (AP)[8]	• Core temperature above 38^0C • Dysuria or frequency in urination	• Flank pain • Pain induced after deep palpation of the right or left costo-vertebral angle • Pyuria defined as more than 10 white blood cells per high power field of spun urine or positive leukocyte esterase in urine; • Ultrasound findings compatible with acute pyelonephritis OR radiological findings from computed urography compatible with acute pyelonephritis

Acute biliary infection (ABI)[9]	• Core temperature above 38^0C • Pain at deep palpation of the upper right quadrant	• Jaundice • Nausea and vomiting • Radiological findings on abdominal ultrasound or computed tomography of the upper abdomen compatible with acute biliary infection
Intrabdominal abscess (IA)[9]	Radiological findings on abdominal ultrasound or computed tomography of the abdomen typical of an intraabdominal abscess	None applies
Secondary peritonitis (SP)[9]	ANY surgical or radiological finding compatible with secondary peritonitis	
Primary bacteremia[9]	• Peripheral blood culture positive for Gram-positive or Gram-negative bacteria or fungal species. • Absence of any primary site of infection after an extensive patient work-out.	None applies