PROSPECTIVE, NON-INTERVENTIONAL, MULTI-CENTRECLINICALSTUDYTOASSESSTHECLINICALVALIDITYOFTHE HEPARINBINDINGPROTEINASSAYTOINDICATE THE PRESENCE AND OUTCOME OF SEPSIS (INCLUDING SEPTIC SHOCK) IN PATIENTS WITH SUSPECTED INFECTION FOLLOWING EMERGENCY DEPARTMENT ADMISSION: THEPROMPT TRIAL.

Clinical Study Protocol

Version: 1

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	is estimated that 80 patients/centre will be enrolled.
Duration	8months

ABBREVIATIONS

APACHE: Acute Physiology and Chronic Health Evaluation

HBP: heparin binding protein

INR: international normalised ratio

qSOFA: quickSOFAscore

SIRS: systemic inflammatory response syndrome

SOFA: Sequential Organ Failure Assessment

INTRODUCTION

The purpose of this prospective, non-interventional, multi-centre clinical study is to assess the clinical validity of the Heparin Binding Protein (HBP) assay for indicating the presence, or outcome, of severe sepsis (including septic shock), over 72 hours, in patients with suspected infection following emergency department admission.

Sepsis is an increasingly common cause of morbidity and mortality, with approximately 150,000 people in Europe and 215,000 people in the US dying of severe sepsis each year[1,2]. Deaths attributable to sepsis continue to rise due to an increase in incidence of the disease,which can be attributed to numerous factors including the aging population, the increased number of immuno-compromised patients, the increased use of invasive surgery and the increased incidence of microbial resistance[3].

The sepsis syndrome was first described in the 1992 publication by Bone et al detailing the conclusions of the ACCP/SCCM Consensus Conference held in 1991, which first introduced the Systemic Inflammatory Response Syndrome (SIRS) classification system[4]. Systemic Inflammatory Response Syndrome (SIRS) is considered to be present when patients have 2 or more of the following clinical findings:

- body temperature, >38ºC or <36ºC
- heart rate, >90 beats per minute
- respiratory rate of >20 breaths per minute or a PaCO2 of <32mm Hg
- white cell count of >12,000 cells per µL or <4,000 per µL, or >10% immature (band) forms

According to the suggested definitions, sepsis is defined as SIRS plus confirmed infection, severe sepsis is defined as sepsis associated with organ dysfunction, hypoperfusion, or hypotension and septic shock is defined as sepsis-induced hypotension, persisting despite adequate fluid resuscitation.

Diagnosis of sepsis traditionally relies on identification of the above symptoms, as well as culturing techniques to confirm and identify the infection. This method of

diagnosis is, however, far from ideal as it has been demonstrated that SIRS criteria are poorly predictive of subsequent events in the sepsis cascade and that approximately one half of severe sepsis cases are culture negative[5,6]. In addition, the assay time for culture-based diagnosis is 24 to 48 hours, where it has been shown that diagnosis of severe sepsis and septic shock as early as possible is important, as each hour of delay in effective antimicrobial administration is associated with an average decrease in survival of 7.6%[7]. Although the majority of severe sepsis patients receive treatment in an intensive care unit (ICU), it is estimated that up to two thirds of those patients initially present to the emergency department (ED), and that approximately 20% of patients with confirmed infection who present to the ED with uncomplicated sepsis progress to severe sepsis or septic shock within 72 hours[8,9].

This high incidence of early progression to severe sepsis and septic shock among patients presenting to the ED highlights the time-sensitive nature of diagnosis, especially in patients who initially do not appear critically ill. Therefore early intervention to prevent subsequent or worsening clinical deterioration is key to the successful treatment of patients[10]. However, two major impediments to the effectiveness of sepsis treatment strategies are a failure to recognise the early stages of the disease and underestimation of its severity, as it is difficult to determine which of the patients with signs of infection on initial evaluation have, or will develop, more serious illness. Several outcome prediction models, including Acute Physiology and Chronic Health Evaluation (APACHE) IV, the Simplified Acute Physiology Score (SAPS) III, the Logistic Organ Dysfunction Score (LODS), and the Mortality Probability Model (MPM) III have therefore been developed for use in clinical practice[11-14]. MoreoverpatientsadmittedintheEDwithatleasttwoofthreeclinicalsigns (hypotension, tachypnea, altered mental status) are highly possible to suffer from sepsis. ThesethreeclinicalsignsaretheqSOFAscore. DatafromtheHellenicSepsisStudyGroupputintoquestioniftheqSOFAscorecanpredictsepsisintheEDwithsensitivitythatexceeds 65% [15, 16]. There is, therefore, still an unmet need for a diagnostic tool that can identify those patients at risk of developing more severe disease, and although a number of laboratory measures or novel sepsis

biomarkers have been proposed for clinical use, there is currently no single accepted biomarker or combination of biomarkers for use in patients with suspected sepsis.

The recent publication by Linder et al[17] has shown that measurement of heparin binding protein (HBP), also known as azurocidin or CAP37, in febrile patients presenting to the ED shows a close correlation between increased plasma HBP levels and the development of severe sepsis with hypotension or shock. In this prospective study of 233 febrile adult patients with suspected infection, 26 were diagnosed with severe sepsis with septic shock, 44 with severe sepsis without septic shock, 100 with sepsis, 43 with infection without SIRS and 20 with SIRS without infection. Using a cut-off of 15ng/mL, HBP showed a sensitivity in diagnosing severe sepsis (with or without septic shock) of 87.1%, a specificity of 95.1%, a positive predictive value (PPV) of 88.4% and a negative predictive value (NPV) of 94.5%, which exceeded those values obtained for the other tested markers. Receiver-operating characteristic (ROC) curves also demonstrated that HBP was the best predictor of severe sepsis, with an area under the curve (AUC) value of 0.95. It is therefore hypothesised that HBP may be used for the early identification of patients at risk of developing severe sepsis and septic shock. In addition, it was observed that 20 of the patients with severe sepsis were monitored with serial plasma sample collection during the course of the disease, and that the 18 of these patients who survived had HBP levels that decreased rapidly when the clinical signs improved and the blood pressures were normalised. It is therefore further hypothesised that a decrease in HBP levels may be used to predict survival.

AIM OF THE STUDY

The primary objective of this study is to use HBP concentration to indicate the presence, or outcome, of sepsis over 72 hours after ED admission.The secondary objectives of this study are to separately evaluate the performance of HBP to predict outcome in patients with suspected infection over 12-24 hours after ED admission.

STUDYDESIGN

This is 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. Patients will be enrolled after written informed consent provided by themselves or provided by first-degree relatives in case of patients unable to consent.
Thestudywillberegisteredinwww.clinicaltrials.govbefore enrollment of the first patient.

Inclusion criteria

- Age ≥18 years
- Male or female
- Written informed consent
- Suspected infection
- PresenceofatleastONEofthefollowing:

Temperature> 38°C
Temperature< 36°C
Heart rate> 90 bpm
Respiratory rate > 20/min
Self reported fever/chills

Exclusion criteria

None

Interventions

If a patient meets all inclusion criteria and none of the exclusion criteria, he/she can be further screened for the study. Atthattimepoint, 10mlofbloodwill becollectedfromhim/herundersterile conditioninoneheparin-coatedtubes. Onesamplewillbedirectlycentrifugedandsupernatant will be stored.

Patientfollow-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.
- Specific recording on day 1 before blood sampling of the three elements of the qSOFA score for patients presenting with infections outside the ICU: 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 and SOFA score. APACHE II score is calculated based on the information provided in APPENDIX I and SOFA score in APPENDIX II.
- Outcome on day 28 (death or survival) with recording of the exact day of death in case of death

STUDYENDPOINTS

Primary endpoint

- SensitivityofHBPforsepsisdiagnosis.Everyvalueover 90% isconsideredsatisfactory.

Secondary endpoints

- Specificity, positivepredictivevalueandnegativepredictivevalue of HBP to diagnose sepsis.
- Specificity, positivepredictivevalueandnegativepredictivevalue of HBP to diagnose septic shock.
- Sensitivity, specificity, positivepredictivevalueandnegativepredictivevalue of HBP to predict unfavorable outcome over 72 hours.
- Sensitivity, specificity, positivepredictivevalueandnegativepredictivevalue of HBP to predict unfavorable outcome after 28 days.

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.

Septic shock is defined as the presence of ALL the following:

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

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.0.90and (1-p)=0.10; and e is the desired precision which is equal to half desired confidence interval (CI) width, hence e=5%/2=0.025.

Substituting the numbers in the equation we get N=350. Taking into consideration that some patients will be excluded from analysis due to incomplete dataset, we assume that a total of 400 patients need to be enrolled. Taking into consideration that there will be five study sites, it is estimated that 80 patients/centre will 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. Sensitivity, specificity, positive predictive value and negative predictive value for all endpoints will be provided as percentage (%) and 95% CIs. Comparisons between patients will be done by the Fisher exact test.

SAFETY

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

STUDY DURATION

It is estimated to eight (8) months.

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APPENDIX I: 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. 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=----------

APPENDIXII: The 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 dopamine	≤1 µg/kg/minnoradrenaline	>1 µg/kg/min noradrenaline
Glascow Coma Scale	15	13-14	10-12	6-9	<6
Creatinine (mg/dl) (orurinary output/day)	<1.0	1.2-1.9	2.0-3.4	3.5-4.9 (<500)	≥5.0 (<200)

MAP: mean arterial pressure