OFFICIAL STUDY TITLE: PHYSIOLOGIC EFFECTS OF STRESS DOSE CORTICOSTEROIDS IN THE MANAGEMENT OF INHOSPITAL CARDIAC ARREST – CORTICA

PROTOCOL SUBMITTED FOR IRB APPROVAL: Evaggelismos General Hospital, Athens, Greece, [Protocol ID: CORTICA 15519/23/5/16]; Date: MAY 23, 2016.

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PHYSIOLOGIC EFFECTS OF STRESS DOSE CORTICOSTEROIDS IN THE MANAGEMENT OF INHOSPITAL CARDIAC ARREST - CORTICA

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INTRODUCTION AND RATIONALE OF THE STUDY

Despite recent improvements in the quality of care, in-hospital cardiac arrest is still associated with a high probability of poor outcome. [1, 2]. Patients resuscitated from vasopressor-requiring cardiac arrest frequently exhibit early postresuscitation hemodynamic instability that is poorly responsive to hemodynamic support with high vasopressor doses and intravenous fluids titrated to cardiac filling pressures of at least 12 mmHg [3, 4]. Furthermore, preceding studies indicate that postresuscitation disease is a "sepsis-like" syndrome characterized by plasma cytokine elevation, endotoxemia, coagulopathy and adrenal insufficiency contributing to postresuscitation shock [5-14]. Steroids are currently being used for improving hemodynamics in septic patients [15]; however, their effect on postresuscitation hemodynamics has not been thoroughly elucidated yet.

OBJECTIVES

To determine whether stress-dose steroid supplementation during and after cardiopulmonary resuscitation (CPR) improves the hemodynamic parameters (arterial blood pressure, cardiac output, cerebral blood flow) in patients with in-hospital cardiac arrest. Furthermore to study the effects of steroid administration on the inflammatory response and organ failures, and to determine potential, corticosteroid- associated complications such as hyperglycemia, infections, bleeding peptic ulcers and paresis. In summary, we aim to directly assess the physiological effects and safety of steroids during and after CPR. The possible clinical usefulness of steroids during and after CPR - in the context of early postresuscitation hemodynamic support - currently corresponds to an important knowledge gap, as recently acknowledged by Guidelines Evidence Reviewers. [16, 17].

METHODS

Setting: Intensive/coronary care units, (ICUs/CCUs) of the Evaggelismos Hospital, Athens, Greece (1,200 beds), and of the Larissa University Hospital, Larissa, Greece (700 beds).

Clarification: Every effort will be made to admit patients to an Intensive Care Area as soon as possible, with an estimated time limit of 24 hours. In cases of inability of ICU/CCU admission within 24 hours of resuscitation, every effort will be made to optimize the patient's treatment conditions. Until ICU/CCU admission, patients will be monitored by ICU doctors for adequate respiratory and hemodynamic support, as well as adequate treatment of any life-threatening pathology. Hemodynamic / respiratory monitoring will include continuous electrocardiogram (ECG - lead II), noninvasive blood pressure every 5 min, pulse oximetry, and arterial blood gas analysis at 20 min, and at 4, 12, and 24 hours (and every 12 hours thereafter)

following the Return of Spontaneous Circulation (ROSC). Therapeutic orders and/or recommendations will ultimately be provided by a senior Intensivist.

PATIENTS

Eligibility criteria: Patients who have experienced an in-hospital, vasopressor-requiring cardiac arrest, according to the Guidelines for Resuscitation from 2015. [16, 17]. Patients should have ROSC for at least 20 minutes.

Exclusion criteria: Age <18 years; and/or "end-stage" disease [survival expectancy <6 weeks - mainly cancer patients with bone and / or cerebral metastases, or metastatic / primary disease causing failure of vital organs, including the respiratory system, Sequential Organ Dysfunction Assessment score ≥15 prior to cardiac arrest and immunocompromised patients with new, sepsis-induced complications]; and/or an existing DNAR (Do not-Attempt-Resuscitation) order; and/or Coordinator Intensivist's estimated inability to admit the patient to an Intensive Care Area within 48 hours of ROSC; and/or any deviation from the hospital's standard resuscitation procedure; and/or uncontrollable hemorrhage (e.g., aortic aneurysm rupture); and/or cardiac arrest before hospital admission; and/or any prior treatment with intravenous corticosteroids; and/or any "positive" history of allergic reaction, or active peptic ulcer; and/or presence of evidence compatible with transmural myocardial infarction; and/or any prior inclusion in or exclusion from the present study. Finally, ROSC at any time prior to epinephrine administration, corresponding to "premature randomization" [18], will result in the patient's exclusion due to the "absence of cardiac arrest requiring vasopressors [4] ".

ETHICS AND INFORMED CONSENT

The study will be conducted in concordance with the European Union Clinical Trials Regulation No 536/2014 and the Helsinki Declaration [19, 20]. Due to the emergency situation, consent will not be requested for steroids supplementation during CPR [3, 4]. The patients' families and patients who regain consciousness and communication ability during follow-up will be informed about the study as soon as possible, and any objection will result in exclusion of the patient data from any subsequent analyses. Informed, written next-of-kin consent and non-written patient consent (whenever feasible) will be requested as soon as possible for stress-dose hydrocortisone in postresuscitation shock and continued participation in the study [3, 4]. If consent cannot be obtained before patient death, the patient's next of kin will be informed of the study and their permission for inclusion of the patient data in the subsequent analyses will be requested – (of course in the absence of fulfilment of any exclusion criteria).

The original protocol version has been approved by the Institutional Review Board (IRB) of Evaggelismos General Hospital on June 14 2016 (Approval No. 126/16-6-2016), and by the IRB of the Larissa University Hospital on October 10, 2016 (Approval No. 46113/11-10-2016 - IRB Discussion No. 13/10-10-2016 Θ.6). Subsequent protocol amendments have been approved by the Evaggelismos IRB on January 24, 2017 (Approval No. 8/26-1-2017), and this has been communicated to the IRB of the Larissa University Hospital. The aforementioned amendments were ratified through Approval No. 1769/17-5-2017 of the IRB of the Larissa University Hospital.

STUDY DESIGN

We propose a prospective, randomized, double-blind, placebo-controlled, parallel-group clinical trial.

Randomization: Research Randomizer version 4 (https://www.randomizer.org/) will be used by the study statistician for group allocation. For each study center, random numbers (range, 1-100) will be generated in sets of 4. Each random number of each set will be unique and correspond to 1 of the consecutively enrolled patients. In each set, an odd or even first number (***for example***) will result in assignment of the corresponding patient to the Control or Steroids group, respectively. In each study center, the group allocation rule will be known solely by the pharmacists who will prepare the study drugs.

CPR AND POSTRESUSCITATION INTERVENTIONS

We will enroll adult in-patients with cardiac arrest due to ventricular fibrillation/pulseless tachycardia not responsive to three direct current countershocks, or asystole, or pulseless electrical activity. Study treatments will be administered during the first CPR cycle postenrollment. Patients will be randomized to receive either methylprednisolone 40 mg (Steroids group) or normal saline placebo (Control group) on the first, postenrollment CPR cycle. Otherwise, advanced life support will be conducted according to the 2015 Guidelines for Resuscitation. After resuscitation, patients will be treated with either stress-dose hydrocortisone of 240 mg daily for 7 days maximum (Steroids group), or saline placebo (Control group). More specifically, at 4 hours after ROSC, patients will receive 100 mL/day (average pump infusion rate ~ 4.2 mL/h) of normal saline that will either contain the stress-dose of hydrocortisone (Steroids group) or solely saline placebo (Control group) for a maximum of 9 days. On days 8 and 9 the hydrocortisone dose of the Steroids group will be tapered to 120 mg and 60 mg, respectively, and finally discontinued on day 10 postrandomization. On ICU/CCU admission, patients will receive a central venous line, and an arterial line either standard or as part of pulsatility index continuous cardiac output monitoring. Patients with a standard arterial line may also receive a pulmonary artery catheter, depending on the materials' availability and attending physicians' judgment.

DOCUMENTATION AND PATIENT FOLLOW-UP

CPR attempts will be documented according to the Utstein style [4, 21]. Hemodynamics and gas-exchange, electrolytes, glucose, core body temperature, lactate, and administered fluids and vasopressor/inotropic support will be determined/recorded during CPR, and at ~20 min and ~4 hours as well as at 24, 48, and 72 hours after ROSC; ROSC will be defined as sustained presence of a palpable arterial pulse for at least 20 min. Postresuscitation cardiac output will be monitored for at least 72 hours post-ROSC, and postresuscitation cardiac function will be assessed by ultrasonography within the first hour after ICU admission and at 72 hours post-ROSC. Central-venous blood gas analysis will also be performed at the aforementioned time points and blood samples will be taken for the determination of cytokines at approximately 20 min and at 4, 24, 48, and 72 hours post-ROSC. Follow- up during the first 10 days postrandomization will include 1) Determination/recording of hemodynamics and hemodynamic support, gas-exchange, fluid balance (of the preceding 24 hours), and arterial blood lactate and central venous oxygen saturation at 9 a.m.; 2) Daily determinations of serum pro-inflammatory cytokines (first 72 hours and day 7), and 3) Daily recording (within 8-9 a.m.) of laboratory data, and prescribed medication. The results of 4 daily determinations (1 every 6 hours) of blood glucose will also be recorded to subsequently analyze the incidence of hyperglycemia (i.e., blood glucose exceeding 200 mg/dL) [3, 4]. Follow-up to day 60 post-ROSC will include organ failure-free days, and ventilator-free days. Morbidity/complications throughout ICU/CCU and hospital

stay, and times to ICU/CCU and hospital discharge will also be recorded.

OUTCOME MEASURES

Primary: 1] Hemodynamic parameters including arterial blood pressure and central venous oxygen saturation at 20 minutes after resuscitation and at 4, 24, 48 and 72 hours after ROSC.
Secondary: 1] Cardiac output at 4, 24, 48 and 72 hours after ROSC. 2] Left and right end-diastolic areas of the right and left ventricle, left ventricular ejection fraction, and eccentricity index within the first 12 hours, and at 72 hours after ROSC. 3] *In a total of 50 patients*: Blood Flow Index (BFI) of the frontal cortex and the vastus lateralis by Near-Infrared Spectroscopy (NIRS) and intravenous Indocyanine Green (ICG) dye at 4 and 72 hours after resuscitation [22]. 4] Body temperature in the first 48 hours after resuscitation. 5] Levels of TNFα, IL-1β, IL-6, IL-8, IL-10, at 4, 24, 48, and 72 hours after randomization. 6] Number of organ failure-free days within days 1 to 60 after randomization. 7] Survival to hospital discharge with good neurological outcome defined as Cerebral Performance Category Score 1 or 2 [4]. 8] Potentially corticosteroid-associated complications such as hyperglycemia, infections, bleeding peptic ulcers, and paresis throughout hospital stay.

STATISTICAL ANALYSIS

Data will be reported as mean ± standard deviation, or median (interquartile range), or number (percentage), unless otherwise specified. Distribution normality will be tested by Kolmogorov-Smirnov test. Dichotomous and categorical variables will be compared by two-sided chi-square or Fisher's exact test. Continuous variables will be compared by two-tailed, independent samples t test or the Mann-Whitney exact *U* test. P-values of multiple t-test comparisons will be subjected to the Bonferroni correction. We will use mixed-model analysis to compare repeatedly measured variables between the two groups. Survival data will be analyzed by a previously employed methodology of multivariable Cox regression. [4]. Based on previously published data on the mean arterial pressure at 24 hours postresuscitation [3, 4], to detect an effect size d of 0.761 with an α error probability of 0.015 and a power of 0.80, we need to enroll a total of 78 patients (39 in each group). A target enrollment of 100 patients with ROSC for at least 20 min will likely adequately compensate for possible dropouts or missing data (or for a possible effect of protocol breaches – please see below).

NOTE

This study will not cause any financial burden to the hospital; determination of secondary outcomes (especially #3 and #5) will be based on the adequacy of external funding.

REFERENCES

1. Girotra S, Nallamothu BK, Spertus JA, et al. Trends in survival after in- hospital cardiac arrest N Engl J Med 2012; 367:1912-1920.
2. Al-Alwan A, Ehlenbach WJ, Menon PR, Young MP, Stapleton RD. Cardiopulmonary resuscitation among mechanically ventilated patients. Intensive Care Med 2014; 40:556–563.
3. Mentzelopoulos SD, Zakynthinos SG, Tzoufi M, et al. Vasopressin, epinephrine, and corticosteroids for in-hospital cardiac arrest. Arch Intern Med 2009; 169:15-24.
4. Mentzelopoulos SD, Malachias S, Chamos C, et al. Vasopressin, steroids, and epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a randomized clinical trial. JAMA 2013; 310:270-279.
5. Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome. Circulation 2002;106:562-568.
6. Adrie C, Laurent I, Monvhi M, Cariou A, Dhainou JF, Spaulding C. Postresuscitation disease after cardiac arrest: a sepsis-like syndrome? Curr Opin Crit Care. 2004;10:208-212.
7. Pene F, Hyvernat H, Mallet V, et al. Prognostic value of relative adrenal insufficiency after out-of-hospital cardiac arrest. Intensive Care Med. 2005;31:627-633.
8. Kim JJ, Lim YS, Shin JH, et al. Relative adrenal insufficiency after cardiac arrest: impact on postresuscitation disease outcome. Am J Emerg Med. 2006;24:684-688.
9. Hékimian G, Baugnon T, Thuong M, et al. Cortisol levels and adrenal reserve after successful cardiac arrest resuscitation. Shock. 2004;22:116-119.
10. Tsai MS, Huang CH, Chang WT, Chen WJ, Hsu CY, Hsieh CC, et al. The effect of hydrocortisone on the outcome of out-of-hospital cardiac arrest patients: a pilot study. Am J Emerg Med. 2007;25:318-325.
11. Schultz CH, Rivers EP, Feldkamp CS, et al. A characterization of hypothalamic-pituitary-adrenal axis function during and after human cardiac arrest. Crit Care Med. 1993;21:1339-1347.
12. Vaahersalo J, Skrifvars MB, Pulkki K, et al; FINNRESUSCI Laboratory Study Group. Admission interleukin-6 is associated with post resuscitation organ dysfunction and predicts long-term neurological outcome after out-of- hospital ventricular fibrillation. Resuscitation. 2014;85:1573-1579.
13. Bro-Jeppesen J, Kjaergaard J, Stammet P, et al; TTM-trial investigators. Predictive value of interleukin-6 in post-cardiac arrest patients treated with targeted temperature management at 33°C or 36°C. Resuscitation. 2016;98:1-8.
14. Peberdy MA, Andersen LW, Abbate A, et al; National Post Arrest Research Consortium (NPARC) Investigators. Inflammatory markers following resuscitation from out-of-hospital cardiac arrest-A prospective multicenter observational study. Resuscitation. 2016. doi: 10.1016/j.resuscitation.2016.01.006. [Epub ahead of print].
15. Dellinger RP, Levy MM, Rhodes A, et al, Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013;39:165-228.

16. Perkins GD, Handley AJ, Koster RW, et al European Resuscitation Council Guidelines for Resuscitation 2015 Section 2. Adult basic life support and automated external defibrillation Resuscitation 2015; 95:81–99.
17. Link MS, Berkow LC, Kudenchuk PJ, et al. Part 7: Adult Advanced Cardiovascular Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132(18 Suppl 2):S444-64.
18. Fergusson D, Aaron SD, Guyatt G, Hébert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. BMJ. 2002; 325(7365):652-654.
19. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310:2191-2194.
20. REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Official Journal of the European Union 2014; L158/1-L158/76.
21. Cummins RO, Chamberlain D, Hazinski MF, et al; American Heart Association. Recommended guidelines for reviewing, reporting, and conducting research on in-hospital resuscitation: the in-hospital "Utstein style." Circulation 1997;95:2213-2239.
22. Boushel, R., Langberg, H., Olesen, J., Nowak, M., Simonsen, L., Bulow, J. & Kjaer, M. (2000). Regional blood flow during exercise in humans measured by near – infrared spectroscopy and indocyanine green. J Appl Physiol 2000; 89: 1868-1878.

CORTICA CHECKLIST FOR MINIMUM RECORDING REQUIREMENTS

During resuscitation

- ◯ Study Box Number
- ◯ Initial rhythm
- ◯ Time of resuscitation team call
- ◯ Time to ALS initiation (min)
- ◯ Time of first shock
- ◯ Time of second shock
- ◯ Intubation time (before or during resuscitation)*
- ◯ Central venous catheter placement (before or during resuscitation)*
- ◯ Time of study drug administration*
- ◯ Total epinephrine dose
- ◯ Arterial blood gas
- ◯ Venous blood gas
- ◯ Arterial pressure (Systolic and Diaastolic)
- ◯ Vasopressors
- ◯ Inotropes (calculate total dose if possible)
- ◯ Other drugs [& corrections of electrolyte disturbances / acid-base status]
- ◯ Total fluids / red blood cell or plasma transfusion
- ◯ Temporary pacing
- ◯ Total number of shocks
- ◯ Reversible cause
- ◯ Total number of CPR cycles
- ◯ ALS duration (min)
- ◯ Resuscitation Outcome-Complications
- ◯ Any comments [always valuable!!!]

*Time or Number of CPR cycle

15-20 min and 4 hours after ROSC

◯ Arterial pressure (Systolic / Diastolic)
◯ Heart rate
◯ Twelve-lead ECG abnormalities
◯ Patient responsiveness to voice
◯ Arterial blood gas
◯ Hemodynamic support (norepinephrine, epinephrine, etc. in µg/kg/min)
◯ Total fluids since CPR initiation until 15-20 min after resuscitation
◯ Temperature
◯ Blood sample
◯ Any comments [always valuable!!!]

Patient history: Each investigator is responsible for collecting the patient's medical history. In this source document, the investigator has to indicate the age, sex, body weight and height, past medical history, past medication, cause of current hospital admission, days in hospital before cardiac arrest, cause of cardiac arrest and any other clinically relevant comment regarding the peri-arrest period. This checklist must be e-mailed to the study coordinator.

Pharmacy Trial Instructions

IT IS THE RESPONSIBILITY OF <u>ALL</u> USERS OF THIS SOP TO ENSURE THAT THE CORRECT VERSION IS BEING USED

Template Reference: Version Number: Author: Implementation date of current version:	1.0

Approved by:	Name/Position: Signature: Date:	
	Name/Position: Signature: Date:	

This Template will normally be reviewed every 2 years unless changes to the legislation require otherwise

Version History Log

This area should detail the version history for this document. It should detail the key elements of the changes to the versions.

Version	Date Implemented	Details of significant changes
1.0		

Pharmacy Trial Instructions

Study Short Title	CORTICA
Study Long Title	PHYSIOLOGIC EFFECTS OF STRESS DOSE CORTICOSTEROIDS IN THE MANAGEMENT OF INHOSPITAL CARDIAC ARREST (CORTICA)

IT IS THE RESPONSIBILITY OF ALL USERS OF THIS SOP TO ENSURE THAT THE CORRECT VERSION IS BEING USED AND THAT THEY HAVE SIGNED THE SIGNATURE LOG IN SECTION 4.2

Staff must ensure that they are adequately trained in this procedure and must make sure that all copies of superseded versions are promptly withdrawn from use unless notified otherwise.

If you are reading this in printed form check that the version number and date below is the most recent one.

Study SOP Reference:	Short study title/Cortica
Version Number:	Enter number
Author:	Enter Author
Implementation date of current version:	Enter date

Approved by:	Name/Position:	Research Pharmacist / Directorate Pharmacist
	Signature:	
	Date:	Enter Date
	Name/Position:	Pharmacy Trial Manager/Senior Pharmacy Technician
	Signature:	
	Date:	Enter Date

This SOP will normally be reviewed every 2 years unless changes to the legislation require otherwise

Version History Log

This area should detail the version history for this document. It should detail the key elements of the changes to the versions.

Version	Date Implemented	Details of significant changes

Contents

- 1. **Introduction, Background and Purpose**

It is a requirement that all Pharmacy study files contain a comprehensive standard operating procedure (SOP) to cover all aspects of running the study within Pharmacy.

- **2. Who Should Use This SOP**

This SOP applies to all members of Pharmacy staff involved in undertaking activities relating to this clinical trial.

- **3. When this SOP Should be Used**

This Standard Operating Procedure (SOP) specifically relates to Cortica study and should be followed when undertaking trial activities.

- **4. Procedure(s)**

This document should be retained within the Pharmacy clinical trial file. The Version History Log must be updated whenever a new version is implemented.

4.1 Contact Details

[The contact details of the following individuals should be obtained and included in this section. Add rows as appropriate]

	Name, address, email and telephone number
CI/PI	
Study Pharmacist	

4.2 Staff Signature Log

Name	Job Title	Signature	Date

4.3 CORTICA Study Summary

Background: Patients resuscitated from vasopressor-requiring cardiac arrest frequently exhibit severe, postresuscitation hemodynamic instability, partly attributable to an impaired adrenal release of hydrocortisone. Also, postresuscitation disease is a "sepsis-like" syndrome characterized by plasma cytokine elevation, endotoxemia, coagulopathy, and adrenal insufficiency/impaired hydrocortisone release contributing to postresuscitation shock. Preceding study results testing combined interventions (i.e. vasopressin and stress dose steroids in addition to epinephrine) are consistent with significant physiological benefits of steroid supplementation during and after cardiopulmonary resuscitation (CPR). We propose to test this hypothesis in a randomized, double-blind, parallel-group, prospective study.
Setting and Participants: Two Greek, tertiary care centers (approximately 2000 beds). One hundred patients with vasopressor-requiring cardiac arrest.
Methods–Intervention: Patients will be randomized to receive either methylprednisolone 40 mg (Steroids group) or saline placebo (Control group) on the first, postenrollment CPR cycle. Otherwise, advanced life support will be conducted according to the 2015 guidelines for resuscitation. Shock after resuscitation will be treated with either stress- dose hydrocortisone (240 mg daily for 7 days maximum and gradual taper; Steroids group) or saline placebo (Control group).
Methods - Endpoints: Primary outcomes will be arterial blood pressure, cardiac output, and central venous oxygen saturation at 20 min, and at 4, 24, 48 and 72 hours after ROSC. Secondary outcomes will include left and right ventricular diastolic area and ejection fraction, blood flow index of the brain, and postresuscitation inflammatory response within the first 72 hours after ROSC; organ dysfunction/failure within days 1-60 postenrollment; survival to hospital discharge with/without favorable neurological recovery; and steroid-related complications throughout in-hospital follow-up.
Expected Results: Steroid- associated benefit with respect to the primary outcomes, consistent with results on secondary outcomes.

The medication will be normal hospital stock, but will be refered to as investigational medicinal product (IMP) for the purpose of the study. CORTICA is a blinded randomized trial.

4.4 Patient Log

Patient ID Number	Patient Name	Hospital number	Date of Birth (DD/MM/YY)	Date of randomization /start of treatment (DD/MM/YY)	Treatment arm (if applicable)	Comments (e.g. discontinuation withdrawal of patient)

In the event that this table is completed during the course of the trial – please use a continuation page from Appendix B.

4.5 Prescription Checking Procedure

All clinical trial prescriptions must be checked by a Pharmacist after being dispensed. The signature of a Pharmacist on a prescription for this trial confirms that the following checking procedures have been undertaken by that Pharmacist.

4.5,1 Prescription checking

A Pharmacist should:

- Read through the checking procedure and sign the staff signature log to confirm this has been done
- Check the study details referring to the trial summary
- Check the prescription as follows:
    - correct prescription is in use refer to current version in file
    - patient addressograph is attached to the prescription (or patient details (Name, DOB, Hospital Number) are completed on the prescription)
    - patient details have been completed on the patient log
    - study prescription is completed in full and signed by the person who dispensed
    - allergies box has been completed on the prescription
    - prescribers signature against the delegation log

4.5.2 Study drug checking

A Pharmacist should:

- Check the study drugs as follows:
    - Appropriate according to the randomization of the patient.
    - drug, strength, dose level and route
    - Treatment number, pack number or container numbers are correct against the prescription.
    - correct number of containers/packs dispensed
    - quantity and expiry date/retest date of the IMP is sufficient for the duration of treatment.

IMP has been labelled correctly

- storage conditions of the drugs dispensed are correct

4.5.3 Drug Accountability checking

A Pharmacist should:

- Check the Drug Accountability records have been completed accurately (and add signature or initials to confirm this) as follows:
  - Master accountability record
  - Subject specific accountability record
  - Prescription Pharmacy use only section

4.6 Dispensing Procedure

4.6.1 Study drugs involved

Methylprednisolone Sodium or Hydrogen Succinate 40 mg Single Dose Vial.
Reconstituted with 1.2 mL bacteriostatic water for injection with benzyl alcohol.
This solution may then be added to indicated amounts of isotonic normal saline solution.

Hydrocortisone Sodium or Hydrogen Succinate 250 mg
Single Dose Vial Reconstituted with 2 mL bacteriostatic
water for injection
This solution may then be added to indicated amounts of isotonic normal saline solution.

Pharmacy signature log

All Pharmacy staff involved in dispensing for a clinical trial must sign the Pharmacy Signature log within this document.

4.6.2 Patient log

Patient information needs to be completed on the patient log specific to this trial .

4.6.3 Prescription

A prescription will need to be prepared by Pharmacy to include the following relevant details for the study:

- ☐ *Study title*
- ☐ *Patients addressograph*
- ☐ *Patients study/trial number*
- ☐ *Drug allergies box*
- ☐ *Details of study drug being prescribed*
- ☐ *Principal Investigator signature (or other investigator named on the delegation log)*
- ☐ *Investigator name (PRINT)*
- ☐ *Date of signature*

Pharmacy use only section to include;

- ☐ *Box to complete Drug batch number and expiry date and any other pharmacy relevant information e.g. randomization if appropriate*
- ☐ *Signature of dispenser and date*
- ☐ *Signature of checker and date*
- ☐ *Signature of person collecting prescription and date and time.*

The signature of the Dispenser on the prescription confirms that the above points have been checked and are complete on the prescription.

The dispensing Technician and checking Pharmacist must check that the Prescriber is authorized to prescribe medication as part of the trial by checking the delegation log held in section 7.3 of the Pharmacy Trial File.

4.6.4 Drugs to dispense

- ☐ Methylprednisolone Sodium or Hydrogen Succinate 40 mg in isotonic saline solution-prefilled syringes [total solution volume = 5 mL].
- ☐ 5 mL isotonic saline solution-prefilled syringes [Saline placebo - Control Group].
- ☐ Hydrocortisone Sodium or Hydrogen Succinate 240 mg in 100 mL isotonic saline solution piggy bags.
- ☐ Hydrocortisone Sodium or Hydrogen Succinate 120 mg in 100 mL isotonic saline solution piggy bags.
- ☐ Hydrocortisone Sodium or Hydrogen Succinate 60 mg in 100 mL isotonic saline solution piggy bags.
- ☐ 100 mL isotonic saline solution piggy bags.

4.6.5 Expiry dates

The batch number and expiry date of the drugs dispensed should be checked and recorded by the dispensing pharmacist on the Drug Accountability Log.

4.6.6 Labelling

The dispensing label to be added during dispensing must include the patient's name and trial number, date of dispensing and the signatures of the persons who dispensed and checked the drugs.

4.6.7 Accountability Records

Master Accountability log

The log heading should contain the following;

- ☐ Trial name
- ☐ Drug name and strength
- ☐ Site name and number
- ☐ Investigator name

The main body of the log should contain the following column's;

- ☐ Date
- ☐ Action (Drug receipt or dispense or return)
- ☐ Drug strength
- ☐ Quantity
- ☐ Batch number
- ☐ Expiry date
- ☐ Dispensed items
- ☐ Dispensed by/received by
- ☐ Checked by (initials)
- ☐ Quantity discarded (initials and date) **STUDY DRUG SOLUTIONS MUST BE STORED AT 2-8 DEGREES CELCIUS AND USED WITHIN 24 HOURS OF PREPARATION TO PREVENT POSSIBLE EFFECTS OF HYDROLYTIC DEGRADATION - SOLUTIONS NOT USED WITHIN 24 HOURS MUST BE DISCARDED**
- ☐ Checked by

Subject Specific accountability log

The log heading should contain the following;

- ☐ Trial name
- ☐ Patient trial/study number
- ☐ Patient name/initials
- ☐ Site name/number
- ☐ Principal Investigator name

The main body of the log should contain the following columns;

- ☐ Date
- ☐ Action (drug receipt or dispense)
- ☐ Dose
- ☐ Drug strength
- ☐ Quantity
- ☐ Batch number
- ☐ Expiry date

☐ Dispensed items, patients name, trial number
☐ Dispensed/receipted by
☐ Checked by (initials)?
☐ Quantity discarded (initials and date)
☐ Checked by ?

4.7 Ordering of Trial Supplies

The normal hospital stock will be used for the study purpose.

4.8 Randomization Procedure

Research randomizer-generated random numbers will be allocated in sets of four. Each random number of each set will be unique and correspond to 1 of the consecutively enrolled patients. In each set an odd or even first number ***(example)*** *will result in assignment of the corresponding patient to the No Steroids or Steroids group respectively. The group allocation rule will be known solely by the study Pharmacists who will prepare and dispense study drugs.*
Methylprednisolone 40 mg in 5 mL isotonic saline or isotonic saline-preloaded 5-ml syringes will be placed in boxes bearing patient codes. At the time of enrollment, a box will be opened, and the drug will be administered according to protocol.

5. Appendix A – Staff Signature Log

The following Pharmacy staff sign to confirm that they have read the current version of this study specific dispensing and checking procedure (Pharmacists are only required to read the study summary and checking procedure).

Name	Job Title	Signature	Date

Duplicate page as necessary

- **Appendix B – Patient Log continuation page**

Patient ID Number	Patient Name	Hospital	Date of Birth (DD/MM/YY)	Date of randomization /start of treatment (DD/MM/YY)	Treatment arm	Comments (e.g. discontinuation or withdrawal of patient)

Duplicate page as necessary

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Date	Temperature °C		Initials of the person collecting data	Comments
	MIN	MAX		

Investigator's Signature________________________

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Date	Temperature °C		Initials of the person collecting data	Comments
	MIN	MAX		

Investigator's Signature________________________ Page………

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Date	Temperature ºC		Initials of the person collecting data	Comments
	MIN	MAX		

Investigator's Signature_________________________

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Date	Temperature ºC		Initials of the person collecting data	Comments
	MIN	MAX		

Investigator’s Signature________________________ Page………

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Date	Temperature ºC		Initials of the person collecting data	Comments
	MIN	MAX		

Investigator's Signature________________________ Page………

STUDY	CORTICA
TEST MATERIAL	Methylprednisolone 40 mg (in 5 mL Normal Saline/) Normal Saline (5 mL) Hydrocortisone (60-240 mg) in 100 mL Normal Saline 100 mL Normal Saline

Standard Operating Procedure for the Recording, Management and Reporting of Adverse Events by Investigators

IT IS THE RESPONSIBILITY OF ALL USERS OF THIS SOP TO ENSURE THAT THE CORRECT VERSION IS BEING USED

Template Reference: Version Number: Author: Implementation date of current version:		1.0
Approved by:	Name/Position: Signature: Date:	
	Name/Position: Signature: Date:	
This Template will normally be reviewed every year unless changes to the legislation require otherwise		

Version History Log

Version	Date Implemented	Details of significant changes
1.0		

ACRONYMS	
AE	Adverse Event
AR	Adverse Reaction
CRF	Case Report Form
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
PI	Principal Investigator
IMP	Investigational Medicinal Product
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SEC	Scientific and Research Ethics Committee
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

Contents

1. PURPOSE

This Standard Operating Procedure (SOP) describes the procedure to be used by the investigator for the recording, management and reporting of Adverse Events (AEs), Adverse Reactions (ARs), Serious Adverse Events (SAEs), Suspected Serious Adverse Reactions (SSARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur in subjects participating in the non-commercial, academic, investigator-initiated, prospective, parallel-group, randomized, double-blind CORTICA study.

2. BACKGROUND

This SOP is written in accordance with Good Clinical Practice (GCP) requirements as previously outlined in Directives 2001/20/EC and 2005/28/EC, and currently supported by the CLINICAL TRIALS REGULATION (EU) No 536/2014.

2.1. DEFINITIONS

The following definitions have been adapted:

Adverse Event

Any untoward medical occurrence in a patient or clinical trial subject administered an Investigational Medicinal Product (IMP) and which does not necessarily have a causal relationship with this treatment.

Therefore, an AE can be any unfavorable or unintended change in the structure (signs), function (symptoms) or chemistry (laboratory data) in a subject to whom an IMP has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction

All untoward and unintended responses to an IMP related to any dose administered.

This definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

Serious Adverse Event and Serious Adverse Reaction

Any adverse event or reaction in a trial subject that:

(a) results in death; or

(b) is life threatening; places the subject, in the view of the investigator, at immediate risk of death from the experience as it occurred (this does not include an adverse experience that, had it occurred in a more severe form, might have caused death); or

(c) requires hospitalization or prolongation of existing hospitalization;

(d) results in persistent or significant disability or incapacity

or

(e) consists of a congenital anomaly or birth defect

Note: in offspring of subjects taking the IMP regardless of time of diagnosis.

Important Safety Issues

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention (medical or surgical) to prevent one of the other outcomes listed in the definition above should also be considered serious. Such events might include:

1. Overdoses (accidental or intentional)
2. Pregnancy (of subject)
3. An alarming adverse experience
4. Specific Adverse events and/or laboratory abnormalities which are listed in the trial protocol as critical to safety evaluations and requiring reporting.

Suspected Serious Adverse Reaction

An adverse reaction that is classed in nature as serious and which is consistent with the information about the medicinal product listed in the relevant reference documentation in the case of a licensed product being used within its licensed dosage and indication or in the Investigator's Brochure (IB) in the case of a licensed product being used outside its licensed dosage and indication.

Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Suspected Unexpected Serious Adverse Reaction

An adverse reaction that is classified in nature as both serious and unexpected.

2.2 OTHER SAFETY ISSUES CONSIDERED TO BE SERIOUS IN THE CLINICA TRIAL.

Events which may materially alter the current benefit-risk assessment of an investigational Medicinal Product (IMP) or which could be sufficient to consider changes in the IMP administration or in the overall conduct of the trial may fall into the category of 'Other Safety Issues' and be considered as serious events which will require reporting to the sponsor in a letter headed Safety Report:

a. An increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction, which is judged to be clinically important,

b. Post-study Suspected Unexpected Serious Adverse Reactions (SUSARs) that occur after the patient has completed a clinical trial and are reported by the investigator to the Sponsor,

c. New events related to the conduct of the trial or the development of the IMPs and likely to affect the safety of the subjects, such as:

1) A Serious Adverse Event (SAE) which could be associated with the trial procedures and which could modify the conduct of the trial,

2) A significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease,

3) A major safety finding from a newly completed animal study

d. Recommendations of the Data Monitoring Committee (DMC), if any, where relevant to the safety of the subjects.

An "Other Safety Issue" can also fall into the category of Urgent Safety Measures. Please refer to the Standard Operating Procedure for the Recording and Reporting of Deviations, Violations, Potential Serious Breaches, Serious Breaches and Urgent Safety Measures.

2.3 SEVERE ADVERSE EVENT OR REACTION

The term “severe” is often used to describe the intensity of an event or reaction (e.g. mild, moderate or severe) and should not be confused or interchanged with the term “serious”.

2.4 KEY RESPONSIBILITIES FOR THE INVESTIGATOR

This section describes the key pharmacovigilance responsibilities of the investigator, further delegation of these responsibilities to other team members must be documented on the trial delegation log.

1. The principal investigator (PI) must further ensure that the team are all familiar with the appropriate use of the IMP(s), as described in the protocol.
2. Adverse Event (AE) Recording: All AEs must be recorded in the medical records (if source data) and/or the patient case report form (CRF), Serious Adverse Event (SAE) forms and AE logs as described in the protocol.
3. AE Assessment: The PI / investigator(s) must assess each event for **seriousness, expectedness and causality** using the appropriate documentation (protocol and safety reference document).
4. Trend/signal analysis: The PI must ensure the AE log is reviewed regularly. This can be performed by the PI alone or reviewed collectively at trial meetings. These reviews need to be documented.
5. SAE Reports: The PI must ensure that initial and follow-up SAE reports are sent to the Scientific and Ethics Committee (SEC), according to the protocol.
6. Confidentiality: The PI must always maintain subject confidentiality.
7. Urgent Safety Measure: The PI / investigator(s) may take appropriate urgent safety measures to protect clinical trial subjects from any immediate hazard to their health and safety. This may be taken immediately. However, following the measure the PI / investigator must follow the SOP on “Deviations, serious breaches and urgent safety measures” .

3. SCOPE OF THIS SOP

This SOP covers the procedures for the recording, management and reporting of all AEs, ARs, SAEs, SSARs and SUSARs that occur in subjects participating in the CORTICA Study. This document further details overdose reports, safety alerts, safety reference document updates, and highlights the key pharmacovigilance responsibilities of the PI. **All pertinent documentation must always be readily accessible by the DMC.**

4. RESPONSIBLE PERSONNEL

The PI and the individual investigators within a trial team are responsible for keeping records of all adverse events that occur in trial subjects as per protocol.

5. PROCEDURE

Please ensure that you are using the most recent SOP version.

5.1 Duration of AE Recording

The protocol must clearly define the duration of AE recording.

5.2 Which AE to record and which Forms to use?

The table below provides guidelines for where to record AE information: The PI may further delegate who within the trial team is responsible for reporting to the Sponsor. This delegation must be performed on whether trial members are qualified to perform the delegated task. This must be authorized in the delegation log.

Type of Adverse Events	Format of Recording Information
All Adverse events	Medical Records
All AEs and SAEs (as per protocol)	AE section of CRF
All SAEs (as per protocol)	AE log
All SAEs (as per protocol)	SAE report form

5.3 Which AE to report to the SEC?

All AEs/ARs that fulfill the criteria for the definition of serious, whether expected or not, need to be reported to the SEC.

5.4 Evaluation of AEs/ARs during the trial

The following documents need to be referred to when assessing any AE in the trial:

- Protocol
- Trial specific Procedure for unblinding (if applicable)

Each AE must be evaluated for **seriousness, causality, severity and expectedness.** The PI must assess the AE as serious as per the definition of an SAE in section 2.

5.5. Evaluation of causality

The PI's / investigator's causality assessment is vital information since the PI / investigator(s) is / are best placed to review how the subject has changed since baseline (before treatment is administered). Every effort must be made by the PI to obtain all the required information to determine whether the AE is related to the trial intervention.

The PI is asked to consider the following before reaching a decision:

- Medical History
- Lack of efficacy/worsening of existing condition
- Study treatment(s)
- Other treatments-concomitant or previous

- Withdrawal of study treatment-especially following study discontinuation/end of study
- Erroneous treatment with study medication (or concomitant)
- Protocol related process
- The PI's / investigator's evaluation of severity

5.6. Evaluation of expectedness

The PI must evaluate whether the event is expected or unexpected against the protocol and the safety reference documents for the trial. An event can be considered as "unexpected" if it adds significant information on the specificity or severity of an expected event.

5.7 How to manage reports

The blind for the PI / investigator and if applicable, for those persons responsible for data-analysis and interpretation of results will be maintained until the trial data is locked.

However, a patient's allocation may need to be unblinded under the following conditions:

1. Emergency unblinding: Patient experiencing an AE and requiring treatment which cannot be given without knowledge of the trial arm the patient was randomized to.
2. SUSAR Unblinding: The SEC requires unblinding for the submission of a SUSAR report.

Further information is provided in the Standard Operating Procedure for the Preparation of a Study Specific Randomization, Blinding and Code Break Standard Operating Procedure".

6. REFERENCES

1. DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Official Journal of the European Communities. 2002; L121/34-L121/43.
2. COMMISSION DIRECTIVE 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products (Text with EEA relevance). Official Journal of the European Union. 2005; L91/13-L91/19.
3. INFORMATION FROM EUROPEAN UNION INSTITUTIONS, BODIES, AND AGENCIES. EUROPEAN COMMISSION. Communication from the Commission — Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') Official Journal of the European Union 2011; C172/1–C172/13.
4. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. World Medical Association. JAMA. 2013; 310:2191-2194.
5. REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Official Journal of the European Union 2014; L158/1-L158/76.

Adverse Events Recording and Reporting Log

All events should continue to be recorded in source data and CRF as per protocol. This log must 1) be kept on site; 2) be readily accessible by the DMC; and 3) be sent to SEC upon request
The PI CANNOT DOWNGRADE THE ASSESSMENT.

Patient trial no	Adverse Event term	IMP Name	Is Event Serious Y/N	Serious 'Type'[1]	Start date - stop date	Causal relationship[2]	Severity Grade*	Expected (Y/N)	Outcome[3]	Date site aware of SAE	Date SAE 1st reported to the SEC

KEY:

[1] **1**=resulted in Death, **2**=life Threatening, **3**=required inpatient or prolonged existing hospitalization, **4**=resulted in persistent or significant disability/incapacity, **5**=resulted in congenital anomaly/birth defect, **6**= Important Medical Event.

[2] **a**= definitely, **b**=probably, **c**=possibly, **d**=unlikely, **e**= not related, **f**=not assessable

* as per approved protocol or as per trial CRF definition

[3] **1**= Resolved, **2** = Resolved with sequelae, **3** = Unresolved, **4**= Worsening, **5** = Fatal, **6**= not assessable

Serious Adverse Event Reporting Form

Protocol No: **Name of PI:** **Name of Site:**	Initial Report ☐ Follow-up Report ☐

FOR THE ATTENTION OF:
DMC / SEC / Pharmacovigilance Manager / Regulatory Advisor

Please complete
Name of Person sending report:

Job title of Person sending report:

Email of Person sending report:

Contact Phone number of Person sending report:

THIS IS AN URGENT REPORT THAT REQUIRES IMMEDIATE ATTENTION ☐

1. SAE Onset Date:_____(dd/mm/yyyy)

2. SAE Stop Date:_____(dd/mm/yyyy)

3. Location of serious adverse event: _____

4. Was this an unexpected adverse event? Yes ☐ No ☐

5. Brief description of participant(s) with no personal identifiers:
   Sex: F ☐ M ☐ Age: ____

6. Brief description of the nature of the serious adverse event (attach description if more space needed): _____

7. Category of the serious adverse event:

   ☐ death – date__/__/__(dd/mmm/yyyy)
   ☐ life-threatening
   ☐ hospitalization-initial or prolonged
   ☐ disability / incapacity
   ☐ congenital anomaly / birth defect
   ☐ required intervention to prevent permanent impairment
   ☐ other:_____

8. Intervention type:

   ☐ Medication or Nutritional Supplement: specify_____
   ☐ Device: Specify: _____
   ☐ Surgery: Specify: _____
   ☐ Behavioral: Specify: _____

9. <u>Relationship of event to intervention</u>:

   ☐ Unrelated (clearly not related to the intervention)
   ☐ Possible (may be related to intervention)
   ☐ Definite (clearly related to intervention)

10. Was study intervention discontinued due to event? ☐ Yes ☐ No

11. What medications or other steps were taken to treat the serious adverse event?

_____

_____

12. List any relevant tests, laboratory data, history, including preexisting medical conditions

_____

_____

13. Type of report:

☐ Initial
☐ Follow-up
☐ Final

14. Full list of medications the patients was receiving at the time of the SAE

Signature of PI / investigator:_____Date: _____

Standard Operating Procedure for the Recording and Reporting of Deviations, Violations, Potential Serious breaches, Serious breaches and Urgent Safety Measures

Template Reference: Version Number: Author: Implementation date of current version:		1.0
Approved by:	Name/Position: Signature: Date:	
	Name/Position: Signature: Date:	
This Template will normally be reviewed every year unless changes to the legislation require otherwise		

ACRONYMS:	
GCP	Good Clinical Practice
CTIMP	Clinical Trial of Investigational Medicinal Product
DMC	Data Monitoring Committee
SOP	Standard Operating Procedure
ISF	Investigator Site File
PI	Principal Investigator
CI	Chief Investigator (Study Chair)
CRF	Case Report Form
SEC	Scientific and Research Ethics Committee
USM	Urgent safety measures
TMF	Trial Master File

Standard Operating Procedure for the Recording and Reporting of (protocol and /or GCP) Deviations, Violations, Potential Serious breaches, Serious Breaches and Urgent Safety Measures

1. PURPOSE

This Standard Operating Procedure (SOP) specifies the overall process and procedure for investigators to follow for the CORTICA study in the event of a protocol and/or Good Clinical Practice (GCP) deviation. Criteria to follow are outlined to assess the impact of the deviation in light of the definition of a potential serious breach and / or an urgent safety measure.

This SOP describes the procedure for the principal investigator (PI) / investigator to record the event and notify the Scientific and Research Ethics Committee (SEC).

2. RESEARCH POLICY

All CORTICA SOPs will be reviewed and approved by the SEC of Evaggelismos Hospital, Athens, Greece; This SEC is directly linked to the National Research Ethics Committee.

3. BACKGROUND

In concordance with the currently applicable European Union Clinical Trials Regulation 536/2014, the Investigator/Institution should only conduct the trial in accordance with the **approved protocol** unless an urgent safety measure must be taken.

The PI / investigator, or a person designated by the PI (in the trial delegation log), should **document and explain any deviation** from the approved protocol.

Definitions used throughout this document

3.1 Protocol Deviation: A deviation is usually an **un-intended** departure from the expected conduct of the trial (protocol, SOPs), e.g. a protocol visits date deviation (a common deviation in clinical trials). These events will be identified by the trial team during trial conduct and must be continually monitored by the chief investigator (CI)/PI and site team.

It is recognized that minor deviations from approved clinical trial protocols and GCP occur commonly in Clinical Trials of Investigational Medicinal Products (CTIMPs). Not every deviation from the protocol will result in a serious breach. Many of these instances are technical deviations that do not result in harm to the trial subjects or significantly affect the scientific value of the reported results of the trial. These cases should be **documented in the case report form (CRF)** and *appropriate corrective and preventative action taken to ensure they do not recur*. Please use the CRF and the PI's **Log of (Protocol and/or GCP) Deviations / Violations / Potential Serious breaches / Serious breaches / Urgent Safety Measures.**

3.2 Violations: A violation can occur when there is a consistent variation in practice from trial protocol, SOPs. A violation can be classified as major if there is a significant occurrence which affects participant safety or integrity of the research. You are required to report to the PI any violation that may impact on the subjects' safety or affects the integrity of the study data.

Examples of this include but are not limited to;

- Failure to obtain informed consent (i.e. no documentation in source data or an Informed Consent form).
- Enrolment of subjects that do not meet the inclusion/exclusion criteria.
- Undertaking a trial procedure not approved by the SEC (unless for immediate safety reasons).
- Failure to report a Serious Adverse Event/Reaction.
- Investigational Medicinal Product (IMP) dispensing/dosing error.

Minor Violation - a violation that does not impact on subjects' safety or compromise the integrity of study data. Examples of this maybe;

Missing original signed consent form (but clearly legible photocopy present)

3.3 Serious Breaches of the protocol and/or GCP

Please consider whether the violation that has occurred on site meets the following definitions. These cases must be reported to the SEC as soon as the PI / investigator has become aware of the event.

(1) The PI of CORTICA shall notify the SEC in writing of any serious breach of-
(a) the conditions and principles of GCP in connection with CORTICA; or
(b) the protocol relating to CORTICA, as amended from time to time.

A "serious breach" is a breach which is likely to affect to a significant degree –
(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial.

3.4 Urgent Safety Measures (Implementing a Protocol Deviation under an emergency)

The PI / investigator may implement a deviation from, or a change of the protocol to eliminate an immediate hazard(s) to trial subjects **without** prior approval from the SEC. This is defined as an Urgent Safety Measure:
The PI / investigator may take appropriate urgent safety measure(s) to protect clinical trial subjects from any immediate hazard to their health and safety. The measures should be taken immediately. However, to meet the legal timelines, **the PI / investigator must inform the SEC in writing immediately and within 24 hours.**
See section 6.13 below for the REPORTING procedures.

4. SCOPE OF THIS SOP

This SOP details the process (for PI / investigators) to follow for the recording and reporting of CORTICA protocol deviations and violations. It describes what consideration must be considered to assess whether the deviations and violations also meet the definition of a **potential serious breach** or **urgent safety measure and the reporting requirements.**

5. RESPONSIBLE PERSONNEL

The site PI has the responsibility to record and report any violations to the SEC within the agreed timeframes and in accordance with this SOP if these are deemed a potential serious breach/urgent safety measure. Deviations need only be documented on site, in the case report form (CRF) and on the PI's Log of (Protocol and/or GCP) Deviations/Violations/Potential Serious breaches/Serious breaches/Urgent Safety Measures and file noted where required. Any corrective and preventative action should also be documented and retained in the site file.

The SEC must consider the following actions:
Receipt and Assessment (i.e. assessment of deviations/violations, isolated/systematic incident, patient(s) harmed or put at risk/data credibility etc.)

- Investigation
- Corrective and Preventative Action (CAPA)
- Reporting to the National Research Ethics Committee
- Trial suspension or Trial termination
- Compliance with a 7-day reporting timescale

If the PI is unsure whether a deviation or violation is a potential serious breach, then please notify the Data Monitoring Committee (DMC) and the SEC as soon as possible and provide as much information as possible.

The DMC and SEC should assess the impact of the breach on the scientific value of the trial; this can be carried out in conjunction with the PI/ chief investigator (CI). If a potential serious breach is identified by a member of the DMC, the DMC should further discuss with the CI/PI in order to clarify the situation and recommend appropriate corrective and preventative action. Furthermore, The DMC must report the serious breach to the SEC.

The regulatory timeline will only commence once the DMC has been notified of an event and has assessed the event as being a serious breach.

6. PROCEDURE

6.1 Identification of deviations, violations and potential serious breaches

The judgment on whether a breach is likely to have a significant impact on the scientific value of the trial depends on a variety of factors e.g. the design of the trial, the type and extent of the data affected by the breach, the overall contribution of the data to key analysis parameters, the impact of excluding the data from the analysis etc.

In addition, it is important that the PI notifies the SEC of what corrective and preventative action has been taken (CAPA) to devise a formal plan of corrective and preventative action.

6.1.1 Deviations

Recording: In the CRF and the deviations and violations log and file noted if necessary.

Reporting: Where a deviation is reoccurring and may result in identification of a serious breach, this should be notified to the PI / CI and the DMC.

Escalation: Corrective and preventative actions should be implemented for deviations. It is recommended that reoccurring deviations be discussed at trial meetings, trigger protocol amendments, and if required, detailed in the clinical study report.

6.1.2 Violations

Recording: In the CRF and the deviations and violations log and file noted if necessary.

Reporting: Violations of GCP, protocol and regulations must be notified to the PI / and the DMC within 2 calendar days of becoming aware of that violation.

Escalation: Corrective and preventative actions (including protocol amendments as appropriate) should be implemented for violations. If the violation is determined to be a potential serious breach, then this would be reported to the SEC within regulatory timelines.
It is recommended that reoccurring violations be discussed at trial meetings and detailed in the clinical study report. **Violations may result in trial suspension by Oversight Authorities.**

A violation may constitute the DMC / SEC to undertake a triggered monitoring visit. **All major violations must be resolved to conclusion.** Depending on the nature of the violation it may constitute a Serious Breach of GCP and further follow up and reporting may be required by the DMC in line with current regulations.

6.2 Procedure for notifying Oversight Authorities of a serious breach

6.2.1. Site team to complete the "Notification of Serious Breaches of GCP or Trial Protocol form (see Appendix 1) all available details pertaining to the breach should be documented on the form.

6.2.2. Completed Notification of Serious Breaches of GCP form to be sent to the DMC and the SEC.

6.2.3. SEC to assess and collate information relating to the potential serious breach and report to the National Research Ethics Committee within 7 calendar days.

6.2.4. Violation / serious breach to be noted on the Log of (Protocol and/ or GCP) Deviations/Violations/Potential Serious breaches/Serious breaches/Urgent Safety Measures

In addition, the PI must log the Potential serious breach in the PI's Log of (Protocol and/ or GCP) Deviations/Violations/Potential Serious breaches/Serious breaches/Urgent Safety Measures.

6.3 Assessment by the DMC / SEC

DMC / SEC to discuss potential serious breach internally through:

Discussion with appropriate team members (e.g. regulatory advisor, pharmacovigilance coordinator)

Assess which relevant GCP, regulatory or protocol section the breach was identified in.

Evaluate whether the breach fulfils the regulatory definition of a serious breach. The SEC may seek clarification from the National Research Ethics Committee on a potential serious breach. All supporting documentation pertaining to the breach must be compiled and submitted to the National Research Ethics Committee within 7 days of assessing the event as a serious breach.

If the PI obtains clear and unequivocal evidence that a serious breach has occurred the default position should be for the SEC to notify the National Research Ethics Committee first, within 7 days, investigate and act simultaneously or after notification. In this case, the SEC should not wait to obtain all the details of the breach prior to notification.

6.4 Corrective and Preventative Actions (CAPA):

The DMC, SEC, and the CI/PI must agree on the appropriate corrective and preventative action to be taken and this should be documented and detailed within the body of the notification report.

6.5 Notification to the National Research Ethics Committee:

The completed form should be sent **within 7 days** of the SEC having assessed an event as a serious breach. It is not necessary to wait until all the information is obtained, updates to the report are acceptable. In such cases, plans should be indicated with projected timelines for completion on follow up reports.

6.6 Follow up reports:

Follow up reports should be made in writing; the serious breaches form can also be used for this, provided that the "follow-up" nature of the report is clearly identified.

6.7 Escalation and dissemination process:

Internally:

The institutional manager(s) of the PI / investigator from the site where the breach took place must be notified of the "notification of serious breach" having been sent to the National Research Ethics Committee and be informed of what CAPA is in

place. The manager(s) may have to inform their quality assurance and senior management if necessary.

Externally:

This will be dependent on the nature of the breach and may include other sites and pharmacies affected, other SECs / National Research Ethics Committees etc.

The breach should be circulated to relevant staff for inclusion of relevant information into the study report or publication.

6.8 Urgent Safety Measure and pertinent notification by a site

Where unexpected events require an urgent modification of a clinical trial, the PI may take urgent safety measures without awaiting prior authorization. If such measures justify a temporary halt of the trial, the PI should apply for a substantial modification before restarting the trial.

REFERENCES

1. DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. Official Journal of the European Communities. 2002; L121/34-L121/43.
2. COMMISSION DIRECTIVE 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of such products (Text with EEA relevance). Official Journal of the European Union. 2005; L91/13-L91/19.
3. INFORMATION FROM EUROPEAN UNION INSTITUTIONS, BODIES, AND AGENCIES. EUROPEAN COMMISSION. Communication from the Commission — Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') Official Journal of the European Union 2011; C172/1–C172/13.
4. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. World Medical Association. JAMA. 2013; 310:2191-2194.
5. REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Official Journal of the European Union 2014; L158/1-L158/76.

8. APPENDICES

Appendix 1 Notification of a Serious Breach form
Appendix 2 Notification Examples

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Appendix 1: Notification of a Serious Breach form

Notification of Serious Breach of Good Clinical Practice or Trial Protocol

Your Name:		**Your Organization:**	
Your Contact Details:		**Date Breach Identified by PI:**	
		Date Breach Notified to National Research Ethics Committee:	
Details of Individual committing breach:		**CORTICA; ClinicalTrials.gov Identifier: NCT02790788**	
Report: Tick appropriately	**Initial Report** ☐	**Follow-up Report** ☐	

Please give details of the breach

Potential impact to patient safety and/or data credibility:

☐ Patient safety	☐ Scientific value / data credibility
☐ Patient confidentiality	☐ NA/None
☐ Approval Issues	☐ Other Non-compliances (specify)
☐ IMP	

Background:

(continue on additional sheets if required)

Other relevant information:
(i.e. study status, site(s), SEC, PI details etc.)
(continue on additional sheets if required)

Please give details of the action taken:

This should include: Any investigations by your institution, the results and outcomes of the investigations (if known or details of when they will be available/submitted), how it will be reported in the final report/publication, the corrective & preventative action implemented to ensure the breach does not occur again.
(continue on additional sheets if required)

Actual impact to patient safety and/or data credibility:

☐ Patient safety	☐ Scientific value / data credibility
☐ Patient confidentiality	☐ NA/None
☐ Approval Issues	☐ Other Non-compliances (specify)
☐ IMP	

SIGNATURE PAGE

Author and Job Title:	
Signature:	
Date:	
Authorized by: **Name and Job Title**	
Signature:	
Date:	

Appendix 2: Notification Examples [Please pay special attention to the last 2 Examples]

Notifier	Breach	Is it considered a Serious Breach?
PI	Dosing error. SEC informed. The PI stated that there were no serious consequences to subjects or data.	No. As no significant impact on the integrity of trial subjects or on scientific validity of the trial.
PI	Patient Information Leaflet and Informed Consent updated. At one trial site this was not relayed to the patients until approximately 2-3 weeks after approval. *More information on the potential consequences of the delay should have been provided.*	Possibly not. If this was not a systematic or persistent problem and if no harm to trial subjects resulted from the delay. Yes, if there was a significant impact on the integrity of trial subjects (e.g. there was key safety information not relayed to subjects in a timely manner etc.).
PI	Visit date deviation. *A common deviation in clinical trials.*	No. Minor protocol deviation, which does not meet the criteria for notification.
PI	Investigator failed to report a single SAE as defined in the protocol (re-training provided).	No, if it did not result in this or other trial subjects being put at risk, and if it was not a systematic or persistent problem. In some circumstances, failure to report a Suspected Unexpected Serious Adverse Reaction (SUSAR) could have a significant impact on trial subjects. Sufficient information and context should be provided for the impact to be assessed adequately.
DMC / SEC	Investigator failed to stop trial medication, in response to a peptic ulcer bleed. This occurred with 3 patients over a one-year period, despite identification by the DMC of the first case. Patients were put at increased risk of death.	Yes
PI	**At 24 hours after resuscitation, attending physician prescribed open label stress-dose hydrocortisone for a 22-year old patient with severe hemodynamic instability (norepinephrine requirement**	**Yes, but there is no way to prevent such attending physician decisions, because they have a strong ethical basis. We cannot prevent any Emergency / Urgent Safety Measure aimed at managing a**

	0.5 μg/kg/min to maintain a mean arterial pressure of 70 mmHg) – the CORTICA-related treatment was immediately discontinued, and patient follow-up was continued according to protocol. The patient's data will be analyzed according to the original patient group allocation (intention to treat principle).	**life-threatening condition. Nevertheless, all such cases will be reported to the DMC and SEC on a monthly basis. Any potential impact on the study results will be discussed at regular trial team meetings and with the DMC and or SEC whenever deemed necessary.**
PI	**At 24 hours after resuscitation, the patient is still in the hospital's ward where he/she experienced the cardiac arrest. Despite duty intensivists' efforts for Intensive Care Unit (ICU) bed availability, there seems to be no prospect of imminent ICU admission, ie, within the subsequent 12-24 hours. The patient's vital signs are being monitored [continuous electrocardiogram, and pulse oximetry, and noninvasive blood pressure once every 5 min], a central venous catheter has been placed, and mechanical ventilatory settings have been adjusted by the duty intensivist.**	**Yes, but on the other hand, every effort is being made to achieve an acceptable quality of monitored care. If admission to the ICU of another hospital becomes possible, then the patient data will be recorded until the time point of patient transfer to the other hospital. Otherwise, efforts aimed at admitting the patient to our ICU will continue until this is achieved.**

Standard Operating Procedure for the Randomization, Blinding, and Study Code Breaking

Template Reference: Version Number: Author: Implementation date of current version:		1.0
Approved by:	Name/Position: Signature: Date:	
	Name/Position: Signature: Date:	
This Template will normally be reviewed every year unless changes to the legislation require otherwise		

ACRONYMS:	
PI	Principal Investigator
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
SEC	Scientific and Research Ethics Committee
SOP	Standard Operating Procedure

**Standard Operating Procedure for the
Randomization, Blinding, and Study Code Breaking**

1. PURPOSE

This Standard Operating Procedure (SOP) describes the procedure that the Princippal Investigator (PI) of the CORTICA study must follow for the **trial Specific** Randomization, Blinding, and Code Break Procedures

2. BACKGROUND

Clinical trials are often blinded to hide the treatment group assignment from participants and investigators (in double-blinded studies) to prevent the unintentional biases of either parties affecting subject data.

In order to protect the wellbeing and safety of the trial subject as required in the principles of Good Clinical Practice (GCP), the coding system for the Investigational Medical Product(s) in blinded trials should include a mechanism that permits rapid identification of the product(s) in case of a medical emergency, but one that does not permit undetectable breaks of the blinding in order to protect the integrity and validity of the data. To ensure this, code break procedures must be clearly established.

At the start of any clinical trial the PI should have a written procedure on the randomization, blinding and process for rapidly identifying a blinded Investigational Medicinal Product, as well as the details of authorized personnel who will have access to unblinded data.

Definitions

Allocation concealment: Is where the person randomizing the patient does not know what the next treatment allocation will be.

Blinding: A procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s).

Block Randomization: Is the arranging of treatment allocations in groups (blocks) that are similar to one another.

Code Break: is also known as breaking the blind. It is the mechanism that permits the rapid identification of the trial treatment in case of a medical emergency but does not permit undetectable breaks of the blinding.

Double-blinding: Where the subject(s), investigators, monitor and in some cases, data analyst(s) are unaware of the treatment assignment(s).

Randomization: The process of assigning trial subjects to treatment or control groups using an element of chance to determine the assignments to reduce bias.

Randomization Code: A unique number or code that is linked via a randomization list to treatment.

Simple Randomization: is a subset of individuals (a sample) chosen from a larger set (a population). Each individual is chosen randomly with equal chance of receiving each treatment.

Unblinding: Is the disclosure of the identity of blinded treatment.

3. SCOPE OF THIS SOP

Randomization, blinding and code break procedures (if applicable).

4. RESPONSIBLE PERSONNEL

Any researcher or member of a study team should follow this SOP. The Principal Investigator (PI) of the trial must review, correct as necessary, sign and date the SOP.

The PI is responsible for training all staff personnel in the trial team to ensure their SOP on randomization, blinding and code breaking is well understood and complied with.

5. PROCEDURE

5.1 Randomization Procedure

Research randomizer-generated random numbers will be allocated in sets of four. Each random number of each set will be unique and correspond to 1 of the consecutively enrolled patients. In each set an odd or even first number **(example)** will result in assignment of the corresponding patient to the No Steroids or Steroids group respectively. **The group allocation rule will be known solely by the study Pharmacists who will prepare and dispense study drugs.**

Methylprednisolone 40 mg in 5 mL isotonic saline or isotonic saline-preloaded 5-ml syringes will be placed in boxes bearing patient codes. At the time of enrolment, a box will be opened, and the drug will be administered according to protocol.

5.2 Blinding

- Please refer to the Pharmacy Randomization SOP.

5.3 Code Breaking

Pertains to:

- **Circumstances** where unblinding of individual can be broken such as in a medical emergency where knowledge of the blinded treatment is necessary, for the treatment of an adverse event, in the event of a SUSAR (Suspected Unexpected Serious

Adverse Reaction) needing expedited reporting, or if requested by the Data Monitoring Committee (DMC).

- For the case of an emergency, the Pharmacy Team provides 24-hour cover to access the code break.
- A pertinent to the code break file note should contain: The date & time, reason for unblinding, name & signature of the person requesting the code break, name & signature of the person breaking the code.
- The PI must inform the DMC and other investigators in writing following a code break, with the reasons for unblinding.
- **Circumstances** where patients will be able to remain on the trial following unblinding include all code breaks on grounds of managing a life-threatening condition. Otherwise, a code break will be considered as a protocol violation – please refer to the pertinent SOP. Protocol violations will be treated case wise, and in conjunction with the DMC and the Scientific and Research Ethics Committee (SEC).
- Details of unblinding after study completion should be provided to the DMC and the study analysts.
- Participants or their next-of-kin should be informed of their blinded treatment allocation, if applicable.

6. REFERENCE

1. REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Official Journal of the European Union 2014; L158/1-L158/76.

THE CEREBRAL PERFORMANCE CATEGORY (CPC) SCORE CHECK LIST

◯ CPC score=5; Criteria of Brain Death fulfilled?

◯ CPC Score=4; Vegetative State – [absence of any interaction with the environment]?

◯ CPC Score=3; Patient dependent on others for daily nutrition and hygiene?

◯ CPC Score=3; Severe disturbance of memory or dementia diagnosed by a psychiatrist?

◯ CPC Score=3; Patient paralyzed?

◯ CPC Score=3; Any Sequential Organ Dysfunction Assessment (SOFA) subscore(s) of ≥1 causing or contributing to dependency on others?

Respiratory system, PaO_2/FiO_2 (mmHg)	**SOFA score**
< 400	1
< 300	2
< 200	3
< 100	4

Nervous system, Glasgow coma scale	**SOFA score**
13–14	1
10–12	2
6–9	3
< 6	4

Cardiovascular system, Mean arterial pressure OR vasopressors required	**SOFA score**
MAP < 70 mm/Hg	1
dop ≤ 5 μg/kg/min or dob (any dose)	2
dop > 5 μg/kg/min OR epi ≤ ;0.1 μg/kg/min OR nor ≤ 0.1 μg/kg/min	3
dop > 15 μg/kg/min OR epi > 0.1 μg/kg/min OR nor > 0.1 μg/kg/min	4

Drug abbreviations: dop for dopamine, dob for dobutamine, epi for epinephrine and nor for norepinephrine.

Liver, Bilirubin (mg/dl) [μmol/L]	**SOFA score**
1.2–1.9 [> 20-32]	1
2.0–5.9 [33-101]	2
6.0–11.9 [102-204]	3
> 12.0 [> 204]	4

Coagulation Platelets $\times 10^3/\mu l$	**SOFA score**
< 150	1
< 100	2
< 50	3
< 20	4

Kidneys, Creatinine (mg/dl) [μmol/L] (or urine output)	**SOFA score**
1.2–1.9 [110-170]	1
2.0–3.4 [171-299]	2
3.5–4.9 [300-440] (or < 500 ml/d)	3
> 5.0 [> 440] (or < 200 ml/d)	4

◯ CPC Score=2; Can the patient perform independent activities of daily life? – Specify / provide examples

◯ CPC Score=2; Does the patient have hemiplegia, seizures, ataxia, dysarthria, dysphasia, or permanent memory or mental changes? – Specify – Is diagnosis confirmed by a neurologist and/or psychiatrist?

◯ CPC Score=2; Any (SOFA) subscore(s) of ≥1? - please see also above

◯ CPC Score=1; Is the patient conscious and alert? – Does he or she seem able to work and lead a normal life?

The Glasgow Pittsburgh Cerebral Performance Category (CPC) scale;

Please check ALL boxes as appropriate.

CPC level	Item	Yes	No
1	Good cerebral performance (i.e., patient is conscious, alert and able to work and lead a normal life)		
2	Moderate cerebral disability (i.e., patient is conscious and has sufficient cerebral function for independent activities of daily life; hemiplegia, seizures, ataxia, dysarthria, dysphasia, or permanent memory or mental changes, and/or noncerebral organ system dysfunction causing moderate disability may be present)		
3	Severe cerebral disability (i.e., patient is conscious and ambulatory but dependent on others, because of severe memory disturbance or dementia, or patient is paralyzed and can communicate only with his/her eyes, as in the locked-in syndrome; severe disability from noncerebral organ system dysfunction can coexist)		
4	Coma/vegetative state (i.e., patient is unconscious and unable of any verbal and/or psychological interaction with the environment);		
5	Death (i.e. certified brain death)		

1st Department of Critical Care Medicine &
Pulmonary Services
GP Livanos and M Simou Laboratories
Medical School of Athens University
Evangelismos Hospital

STANDARD OPERATING PROCEDURE

Written By			
Name	**Function**	**Date**	**Signature**
Vassiliki Karavana	Lab Manager		

Verification			
Name	**Function**	**Date**	**Signature**
Prof. S. Zakynthinos	Head of the Dept		

Plasma Isolation from Human Peripheral Blood

PURPOSE

To isolate plasma from whole blood using centrifugation

PERSONNEL

All staff trained in molecular biology methods

Equipment and Accessories

Laminar flow hood
Centrifuge (eppedorf 5804R)
Pipettes 10-1000 µl (Gilson)
1.5 ml sterile eppedorf tubes endotoxin free
Disposable sterilize tips
Racks
Disposable gloves
Small ice bucket

METHOD

Invert gently the vacutainer tubes once to mix
Place the tubes into a centrifuge and spin at 2,000 xg for 10 minutes at 4^0C (program N)

Remove the tubes from the centrifuge
Open the tubes and aliquot in 1.5 ml eppendorf using pipette. Be careful not to withdraw any of the white interfacial layer
Label the tubes and store at -80^{0}C.
Discard the pellet
Wipe relevant lab surfaces and lab equipment with a solution of 10% bleach (90% water)

DOCUMENTATION

Fill in the patient form
Patient name -Protocol code
Date and time of blood collection
Number and volume of aliquots prepared into -80ºC

RISK ASSESMENT

All handlings of blood samples should be done in a biohazard safety cabinet
Gloves, lab coat and eye protection must always be worn.
Put all blood-related items, gloves used in working with blood into a biohazard waste container located under the hood.
Dispose tips in a biohazard container.
Taking off gloves, wash hands vigorously with water and disinfecting soap
For additional safety information, refer to the risk assessment, hazard data sheets and the Departmental policy.

1st Department of Critical Care Medicine & Pulmonary Services
GP Livanos and M Simou Laboratories
Medical School of Athens University
Evangelismos Hospital

STANDARD OPERATING PROCEDURE

Written By			
Name	**Function**	**Date**	**Signature**
Spyros D. Mentzelopoulos	Principal Investigator		

Verification			
Name	**Function**	**Date**	**Signature**
Spyros G. Zakynthinos	Head of the Dept And Study Chair		

Near Infrared Spectroscopy (NIRS) assessment of Cerebral Autoregulation and of Cerebral (Frontal Cortex) Blood Flow

PURPOSE

To assess Cerebral Autoregulation and Cerebral Blood Flow

PERSONNEL

Spyros D. Mentzelopoulos; Sotirios Malachias; Spyros Zakynthinos; Zafeiris Louvaris.

Equipment and Accessories

NIRS monitor; NIRO-200, HAMAMATSU, Photonics KK- Japan,
Two sets of NIRS Transmitter and Receiver Probes and 2 black-colored, elastic cases, each one specifically designed to accommodate a Transmitter and a Receiver Probe.
Sterile Gauzes and disinfectant solution appropriate for skin cleansing and prepping
3M™ Tegaderm™ Transparent Film Dressing with Border [size: 10.0 cm x 15.5 cm]
Medical Adhesive Tape,
Ruler for the measurement of distances in cm,
Disposable gloves,
Laptop Personal Computer (PC) with NIRO200ICG software [HAMAMATSU, Photonics KK- Japan] installed,

METHOD

1. Carefully clean/prep the skin of the forehead and the skin area overlying the right vastus lateralis within 5 to 15 cm of the patella.
2. Place a set of NIRS Transmitter and Receiver probes at either side of the midline of the forehead; approximate distance from eyebrows, 15-20 mm. Interoptode distance should be 40 mm and the imaginary line connecting the centers of the optodes should be perpendicular to (and bisected by) the midline of the forehead.
3. Place a set of NIRS Transmitter and Receiver over the skin overlying the vastus lateralis at 10 cm proximally to the upper rim of the patella. Interoptode distance should be 40 mm and the midpoint of the imaginary line connecting the centers of the optodes should be 10 cm proximal to the upper rim of the patella.
4. Cover the elastic optode cases with gauzes and firmly secure them using tegaderm dressing and adhesive tape.
5. Connect the frontal set of optodes to Channel 1 of the NIRS monitor and the vastus lateralis set of optodes to Channel 2 of the NIRS monitor.
6. Initiate monitor display of NIRS variables and open the "N200ICG.exe" in the laptop PC.
7. Record variables for 10 min (sampling rate, 6 Hz); also record baseline mean arterial pressure/heart rate data and vasopressor and/or sedative/anesthetic drug infusion rates [acceptable mean arterial pressure values: 70-110 mmHg].
8. Press "Event" at the NIRS monitor and infuse 5mg (1 mL solution) of indocyanine green (ICG); concurrently record mean arterial pressure/heart rate data and vasopressor and/or sedative/anesthetic drug infusion rates [acceptable mean arterial pressure values: 70-110 mmHg].
9. Continue recording of NIRS variables for another 35-40 min.
10. Press "Event" at the NIRS monitor and adjust vasoactive drugs to increase or decrease mean arterial pressure by at least 15-20% [acceptable mean arterial pressure values: 70-110 mmHg].
11. Record mean arterial pressure/heart rate data for 10-15 min, i.e. until the new, "desired" mean arterial pressure level is reached and maintained for at least 3 min.
12. Press "Event" at the NIRS monitor and infuse 5mg (1 mL solution) of indocyanine green (ICG); concurrently record mean arterial pressure/heart rate data and vasopressor and/or sedative/anesthetic drug infusion rates [acceptable mean arterial pressure values: 70-110 mmHg].
13. Record NIRS variables for another 20-25 min.
14. Store data as both "OD2" and "txt" files and switch off PC/NIRS monitor; remove optodes; and conclude cerebral autoregulation protocol.
15. Autoregulation will be assessed by using tissue oxygenation index values and concurrently recorded MAP values in a regression analysis, and will be considered as adequate if the pertinent Pearson correlation coefficient is lower than 0.3.[1]
16. Cerebral blood flow and vastus lateralis blood flow will be determined by determination of the respective blood flow indexes after a bolus injection of 5 mg of ICG (2-4).

DOCUMENTATION

Hard copy-recorded data.
Fill in electronic patient form.

RISK ASSESMENT

Any adverse reaction to ICG to be recorded / reported as study protocol-related serious adverse event. Any other complication during the aforementioned NIRS determination of the cerebral autoregulation/blood flow will be accordingly evaluated / reported.

REFERENCES

1. Ono M, Brady K, Easley RB, et al. Duration and magnitude of blood pressure below cerebral autoregulation threshold during cardiopulmonary bypass is associated with major morbidity and operative mortality. J Thorac Cardiovasc Surg. 2014; 147(1):483–489.
2. Boushel R, Langberg H, Olesen J, et al. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. J Appl Physiol 2000;89: 1868-1878.
3. Guenette JA, Henderson WR, Dominelli PB, et al. Blood flow index using near-infrared spectroscopy and indocyanine green as a minimally invasive tool to assess respiratory muscle blood flow in humans. Am J Physiol Regul Integr Comp Physiol 2011;300: 984-92, 2011.
4. Habazettl H, Athanasopoulos D, Kuebler WM, et al. Near-infrared spectroscopy and indocyanine green derived blood flow index for noninvasive measurement of muscle perfusion during exercise. J App Physiol 2010;108: 962-967.

1st Department of Critical Care Medicine &
Pulmonary Services
GP Livanos and M Simou Laboratories
Medical School of Athens University
Evangelismos Hospital

STANDARD OPERATING PROCEDURE

Written By			
Name	**Function**	**Date**	**Signature**
Spyros D. Mentzelopoulos	Principal Investigator		

Verification			
Name	**Function**	**Date**	**Signature**
Spyros G. Zakynthinos	Head of the Dept And Study Chair		

Echocardiographic Assessment of right ventricular (RV) and left ventricular (LV) performance after the return of spontaneous circulation (ROSC)

PURPOSE

To assess RV and LV function within 72 hours of ROSC

PERSONNEL

Aikaterini Megalou; Fotini Lagiou; Panagiotis Politis.

Equipment and Accessories

Vivid 3 Expert machine (General Electric Healthcare, Aurora, OH, USA)
Vivid 7 Expert machine (General Electric Healthcare, Aurora, OH, USA)
3-MHz transducer

METHOD

1. Within 12 hours of randomization: Obtain four-chamber views of the heart in order to determine the RV end-diastolic area (RVEDA), and the LV end-diastolic area (LVEDA).
2. Within 12 hours of randomization: Determine the left ventricular ejection fraction by the "area-length" method.[1]
3. Within 12 hours of randomization: Obtain parasternal short-axis views of the heart to determine the Eccentricity Index, i.e. the ratio of the LV anteroposterior to the LV septolateral diameter, measured at end systole and end diastole.
4. Repeat the aforementioned measurements at 72 hours after randomization.

DOCUMENTATION

Hard copy-recorded data.
Add the data to the dedicated boxes of the electronic patient case report form.
Patient name - Protocol code.

RISK ASSESMENT

No adverse event is expected due to the transthoracic echocardiographic examination of the heart.

REFERENCES

1. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiograph. J Am Soc Echocardiogr. 2005;18:1440–63.

Patient's initials:
Patient's number:

CONSENT DECLARATION OF THE PATIENT'S LEGAL REPRESENTATIVE

PHYSIOLOGIC EFFECTS OF STRESS DOSE CORTICOSTEROIDS IN THE MANAGEMENT OF INHOSPITAL CARDIAC ARREST (CORTICA)

https://clinicaltrials.gov/ct2/show/NCT02790788?term=steroids+and+cardiac+arrest&rank=2

Please write down in the following gap the physician's name who informed you about the study and MARK the following answers.

1. I have read the information leaflet.	YES / NO
2. I was given the opportunity to ask questions and discuss issues pertinent to the study with the physician:	YES / NO
3. I was given satisfactory answers and sufficient information for all my questions.	YES / NO
4. I am aware of my right to withdraw my consent for the participation of my relative in the study at any time and without any obligation to explain the reasons.	YES / NO
5. I was given information about compensation.	YES / NO
6. I understand that by my signature, I authorize the access to and the liberation of the study data of my relative to persons authorized by the study's physician, responsible authorities, and the Independent Ethics Committee, as needed. I understand that at any time I can withdraw my authorization for the use of the study data of my relative. Do you agree to provide your consent so that the aforementioned persons can access the files of your relative?	YES / NO
7. Did you have enough time to reach your decision?	YES / NO
8. Do you agree with the participation of your relative in this clinical trial?	YES / NO

PATIENT'S NAME: (INITIALS) _____

LEGAL REPRESENTATIVE'S NAME: (INITIALS)

ADDRESS:	**SIGN AND DATE**
PHONE:	
INVESTIGATOR'S NAME: (INITIALS)	
ADDRESS:	**SIGN AND DATE**
PHONE:	

STUDY INFORMATION SHEET

PATIENT INITIALS:
PATIENT CODE NUMBER:

MAIN INVESTIGATORS, Evangelismos Hospital: Spyros Mentzelopoulos, Ch Vrettou, Sotirios Malachias, Helen Ischaki, Spyros Zakynthinos. University Hospital of Larissa: Demosthenes Makris, Epaminondas Zakynthinos.

STUDY TITLE: PHYSIOLOGIC EFFECTS OF STRESS DOSE CORTICOSTEROIDS IN THE MANAGEMENT OF INHOSPITAL CARDIAC ARREST (CORTICA)

INTRODUCTION

We request your consent for your family member's participation in this scientific study. The has been approved by our Institutional Review Board (IRB – Scientific Committee). To decide whether you agree (or disagree) with your relative's participation in this study you should fully understand the pertinent risks and benefits. You are asked to read this text and discuss anything you do not understand with the study investigators, or other medical personnel of the Department of Intensive Care Medicine, or any other competent doctor who enjoys your confidence. If you understand the study, you will be asked to sign and date the consent form. If you choose your relative's participation in the study, you will be given a copy of the signed consent form.

CONSENTING FOR YOUR RELATIVE'S PARTICIPATION CONSTITUTES A FREE AND RESPONSIBLE CHOICE OF YOURS.

Your relative may participate or discontinue his/her participation in the study at any time, according to your decision, without in any way losing the advantages of scientifically sound medical care based international guidelines and available medical literature evidence.

If desired, the principal investigators of the study or your relative's attending physician will contact your relative's family physician to inform him/her about the study.

PURPOSE OF THE STUDY

Despite recent improvements in the quality of care, the probability of poor outcome after in-hospital cardiac arrest (death or survival to hospital discharge with severe brain damage) remains high (about 80%) (1). The probability of por outcome is even higher (90-95%) among postresuscitation patients who require mechanical ventilation (2), and among those treated with vasopressors (e.g. epinephrine and / or vasopressin) during cardiopulmonary resuscitation (CPR) (3, 4). Therefore, there remains an urgent need for substantial improvements in the medical management of patients resuscitated from cardiac arrest (5-7).

In previous single center (3) and a recent, three-center prospective, randomized, double-blind study (4), the combination of VSE (Vasopressin-Steroids-Epinephrine) proved superior to Epinephrine alone with respect to the survival to hospital discharge

(3), and discharge with good neurological outcome (4). Although the VSE combination VSE did not enable determination of the relative contribution of vasopressin and steroids on the observed positive results (4), the results of a subsequent (*post hoc*) statistical analysis and sensitivity analysis (4) were consistent with the hypothesis that the administration of stress-dose steroids may be associated with reduced risk of poor outcome (death or survival to hospital discharge with severe neurological disabilities) (2, 4, 8). Furthermore, administering at least one dose of hydrocortisone in patients with postresuscitation shock was associated with a decreased likelihood of poor outcome during in-hospital follow-up compared to control group patients who did not receive steroids (4).

The objective of this research is to study the effect of low-dose (stress-dose) corticosteroids on the hemodynamic status after the restoration of spontaneous circulation and of the postresuscitation systemic inflammatory response. More specifically, we will study the addition of 40 mg of methylprednisolone to standard treatment during CPR, and the use of stress-dose hydrocortisone in the treatment of postresuscitation shock.

INVESTIGATIONAL INTERVENTIONS

Your family member has suffered a cardiac arrest from which he/she has been resuscitated after having received standard treatment or the above-described study intervention. You are being informed about the current study at this particular time point and after the (possible) administration of 40 mg of methylprednisolone during CPR, because of the previously extremely emergent situation of your relative's medical status (i.e., cardiac arrest). This is thoroughly consistent with the Declaration of Helsinki [2013 World Medical Association Declaration of Helsinki] and the current European Clinical Trials Regulation No 536/2014, and this specific Informed Consent Procedure has already been adopted.by prior trials (3, 4, 9-11).

This study includes adult patients with cardiac arrest who did not respond to three consecutive countershocks (according to indications), or asystole or pulseless electrical activity. Patients are being randomized to receive either 40 mg methylprednisolone (steroid group) or normal saline placebo (control group) during the first CPR cycle after study (cycle time duration: about 3 minutes). For the first 30 minutes of CPR the vasopressor regimen will include epinephrine (1 mg); vasopressin (currently unavailable) may be added if (and when) it becomes available (3, 4, 12, 13).

Postresuscitation shock is treated either with hydrocortisone [240 mg daily up to 7 days followed by gradual taper over the next 2 days, and then, discontinuation (3, 4, 14-16)], or placebo (control group). Specifically, at 4 hours after the return of spontaneous circulation (ROSC), patients with hemodynamic instability receive 100 mL / day (average pump infusion rate ~ 4.2 mL / hour) of a normal saline solution either containing (steroid group) or not containing (control group) the aforementioned stress-dose of hydrocortisone (3, 4, 14-16). On days 8 and 9, the dose of hydrocortisone (steroid group) is decreased to 120 mg and 60 mg, respectively, and finally stopped on day 10 after randomization.

If ROSC is not achieved after 10 CPR cycles, corresponding to at least 5 doses of vasopressors, the CPR is continued (at the discretion of the leader of the resuscitation team) with 1 mg of epinephrine per 1-2 CPR cycles or approximately 1 mg of epinephrine every three to five minutes.

The only modification compared to conventional CPR protocols (13) pertains to the use of methylprednisolone in the treatment of the cardiac arrest-associated adrenal insufficiency. In cases of postresuscitation shock, patients who received methylprednisolone according to the study's randomization rule receive stress doses of hydrocortisone exactly as described in the IRB-approved study protocol. Please note that the aforementioned investigational interventions will be mandatorily canceled whenever an attending physician decides to prescribe stress-dose hydrocortisone for postresuscitation shock (3, 4, 14-16).

WE REQUEST YOUR CONSENT FOR THE FOLLOWING CASES:

1) USE OF STRESS-DOSE HYDROCORTISONE FOR CARDIAC ARREST-ASSOCIATED CIRCULATORY SHOCK REQUIRING THE USE OF VASOPRESSORS AND FLUIDS TO MAINTAIN THE PERFUSION OF THE PATIENT'S VITAL ORGANS (3, 4, 14-16) AND
2) BLOOD SAMPLING [OVER THE FIRST 3 DAYS, AND DAY 7] FOR THE EVALUATION OF SYSTEMIC INFLAMMATORY RESPONSE THAT IS USUALLY OBSERVED AFTER SUCCESSFUL CPR.
3] ASSESSMENT OF CEREBRAL BLOOD FLOW (BLOOD FLOW INDEX - BFI) WITH THE METHOD OF NEAR INFRARED SPECTROSCOPY (NIRS) IN CONJUNCTION WITH THE DYE INDOCYANINE GREEN (ICG) AT 4 HOURS (WITH ALLOWANCE UP TO 12 HOURS) AND AT 72 HOURS AFTER ROSC [17]. THIS CONCERNS ONLY PATIENTS WITH NO HISTORY OF ANY ALLERGIC REACTION.
4] ULTRASOUND MONITORING OF THE HEART.

POTENTIAL RISKS FROM STUDY INTERVENTIONS AND THEIR PREVENTION
Potential Hazards: These pertain to corticosteroid side effects, such as susceptibility to infections, digestive tract hemorrhage, healing disorders, hyperglycemia, muscle weakness and myopathy, and transient disorders of orientation, thinking, and behavior. The probability of any allergic reaction associated with a study intervention is considered as close to zero for patients without a history of allergy. Prevention-Control: According to data from extensive analyses of prior studies (18-20), we do not expect an increased incidence of adverse events associated with the protocol; the administered dose of hydrocortisone is low, and the treatment is of limited duration (particularly in patients with increased healing requirements), while patients will be receiving appropriate antibiotic therapy, ulcer prophylaxis, and insulin. Patients will undergo continuous hemodynamic monitoring and will have (at minimum) daily determination of their hematocrit and hemoglobin concentration. The low dose of hydrocortisone is exclusively targeted at the very likely postresuscitation adrenal insufficiency and the associated severe hemodynamic instability (3, 4, 14-21). Patients with a diagnosis of transmural myocardial infarction or with a peptic ulcer will not be included in the study. The protocol will also be suspended in cases of poorly regulated blood glucose, or whenever attending physicians opine that an awake patient has developed steroid treatment-associated confusion / delirium.

EXPECTED BENEFITS
For the patient involved: rapid clinical improvement after resuscitation (3, 4).
For Resuscitation Science: filling of important knowledge gaps and improvement of

clinical practice.

INTERRUPTION OF PARTICIPATION IN THE STUDY
Doctors (attending or principal investigator and attending physician) have the right (and obligation) to terminate your relative's participation in this study without your consent, in case of any unexpected and potentially harmful (to your relative) event.

The participation of your relative in this study is completely voluntary and you may discontinue it at any time. You will be timely informed about the time of study termination. You will be timely informed about the clinical course of your relative throughout the study's follow-up period.

COMPENSATION IN CASE OF INJURY RELATED WITH INVESTIGATIONAL INTERVENTIONS
In case of a study protocol-related complication, the responsible researchers will inform you about the complication, the potential for complication reversal, and about your relative's compensation.

USE OF MEDICAL INFORMATION, PRIVACY AND AUTHORIZATION
All data collected will be safe-guarded for the protection of medical confidentiality. Your relative will be referred to only by initials and a code number. The study information may be used in study reports or scientific presentations.
Additional scientific information of the study (eg, values of variables resulting from measurements of the protocol) will not be recorded in the patient's file. This information will be entered electronically by researchers and will be protected by a password and antiviral computer programs. If desired, the researchers will provide you with a pertinent study information note.

By signing the consent form you permit the aforementioned persons to take the above actions. There will be no publication or communication of the data that reveal your relative's identity. The withdrawal of your relative from the study does not automatically cancel the use of his-her personal information. If you wish to cancel the use of your relative's data you should provide a written request to the responsible investigators, who will then be obliged to respond (to your request).

YOU UNDERSTAND THAT YOU HAVE THE RIGHT OF ACCESS TO THE MEDICAL RESEARCH RECORDS OF YOUR RELATIVE IN ACCORDANCE WITH THE LAW.

REFERENCES

1. Girotra S, Nallamothu BK, Spertus JA, et al. Trends in survival after in- hospital cardiac arrest N Engl J Med 2012; 367:1912-1920.

2. Al-Alwan A, Ehlenbach WJ, Menon PR, Young MP, Stapleton RD. Cardiopulmonary resuscitation among mechanically ventilated patients. Intensive Care Med 2014; 40:556–563.

3. Mentzelopoulos SD, Zakynthinos SG, Tzoufi M, et al. Vasopressin, epinephrine, and corticosteroids for in-hospital cardiac arrest. Arch Intern Med 2009; 169:15-24.

4. Mentzelopoulos SD, Malachias S, Chamos C, et al. Vasopressin, steroids, and epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a randomized clinical trial. JAMA 2013; 310:270-279.

5. Neumar RW, Nolan JP, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation, (American Heart Association, Australian and New Zealand Council on Resuscitation, European Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Asia, and the Resuscitation Council of Southern Africa); the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. Circulation 2008; 118: 2452-2483.

6. Chalkias A, Xanthos T. Pathophysiology and pathogenesis of post- resuscitation myocardial stunning. Heart Fail Rev. 2012; 17:117-128.

7. Chalkias A, Xanthos T. Post-cardiac arrest brain injury: pathophysiology and treatment. J Neurol Sci. 2012; 315:1-8.

8. [No authors listed] A randomized clinical trial of calcium entry blocker administration to comatose survivors of cardiac arrest. Design, methods, and patient characteristics. The Brain Resuscitation Clinical Trial II Study Group. Control Clin Trials. 1991;12:525-545.

9. Wenzel V, Krismer AC, Arntz HR, et al. A comparison of vasopressin and epinephrine for out-of-hospital cardiopulmonary resuscitation. N Engl J Med 2004; 350: 105-113.

10. Stiell IG, Hébert PC, Wells GA, et al. Vasopressin versus epinephrine for inhospital cardiac arrest: a randomized controlled trial. Lancet 2001;358:105-109.

11. Gueugniaud PY, David JS, Chanzy E, et al. Vasopressin and epinephrine vs. epinephrine alone in cardiopulmonary resuscitation. N Engl J Med 2008; 359(1):21-30.

12. Layek A, Maitra S, Pal S, Bhattacharjee S, Baidya DK. Efficacy of vasopressin during cardio-pulmonary resuscitation in adult patients: A meta- analysis. Resuscitation. 2014 pii: S0300-9572(14)00447-X. doi: 10.1016/j.resuscitation.2014.03.303.

13. Link MS, Berkow LC, Kudenchuk PJ, et al. Part 7: Adult Advanced Cardiovascular Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132(18 Suppl 2):S444-64.

14. Keh D, Boehnke T, Weber-Cartens S, et al. Immunologic and Hemodynamic Effects of “Low-Dose”Hydrocortisone in Septic Shock A Double-Blind, Randomized, Placebo-controlled, Crossover Study Am J Respir Crit Care Med 2003;167: 512-520.

15. Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome. Circulation 2002;106:562-568.

16. Annane D, Sébille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002;288:862-871.

17. Boushel, R., Langberg, H., Olesen, J., Nowak, M., Simonsen, L., Bulow, J. & Kjaer, M. Regional blood flow during exercise in humans measured by near – infrared spectroscopy and indocyanine green. J Appl Physiol 2000;89:1868-1878.

18. Moran JL, Graham PL, Rockliff S, Bersten AD. Updating the evidence for the role of corticosteroids in severe sepsis and septic shock: a Bayesian meta- analytic perspective. Crit Care. 2010;14:R134.

19. Annane D, Bellissant E, Bollaert PE, et al. Corticosteroids in the treatment of severe sepsis and septic shock in adults: a systematic review. JAMA 2009 10;301:2362-2375.

20. Minneci PC, Deans KJ, Eichacker PQ, Natanson C. The effects of steroids during sepsis depend on dose and severity of illness: an updated meta-analysis. Clin Microbiol Infect 2009;15:308-318.

21. Yang S, Zhang L. Glucocorticoids and vascular reactivity. Curr Vasc Pharmacol. 2004;2:1-12.