STUDY PROTOCOL

PROTOCOL TITLE:
DetectIon of Severe Sepsis In PATients with neurological haemorrhage (The DISSIPATE Study)

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1

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1. Rationale and Background

Rationale

Systemic inflammatory response syndrome (SIRS) and pyrexia are frequent conditions after brain injuries making conventional detection of sepsis extremely inaccurate[1,2]. Early detection of sepsis helps in reducing the secondary injury to brain and the same time accuracy in detection of sepsis is needed to avoid indiscriminate antimicrobial usage and further antimicrobial resistance. Current commonly used biological markers of sepsis like white cell count (WBC) and CRP (C-Reactive Protein) is unspecific for sepsis in this population as they are usually elevated due to the surgery or trauma. Other biological markers such as procalcitonin (PCT), tumour necrosis factor (TNF) and Interleukin-6 have been evaluated but the costs can be prohibitive, delayed due to the processing time and non-specific[2]. Pelinka *et al* has also showed that traditional sepsis markers like CRP and PCT are inaccurate in head injuries[3,4]. Microbiological culture of bacteria is currently the gold standard for sepsis but processing time takes at least 24 hours and is not specific and is frequently at risk of contamination if not sampled correctly. The indiscriminate use of broad-spectrum antibiotics promotes the selection of antibiotic resistance and has further complications. At the same time, patients presenting with a Sequential Organ Failure Assessment (SOFA) score of 2 or more (which is the new definition of sepsis) have an overall mortality risk of 10% with presumed infection which is greater than overall mortality of 8.1% for ST- segment elevation myocardial infarction[5]. So, clinicians have daily difficulties in deciding if a patient is septic when faced with critically ill patients especially with brain injuries and SIRS.

Background

Activated Partial Thromboplastin Time (APTT) clot waveform analysis (CWA) and WBC activation analysis by flow cytometry-based method are new and very low cost biological tests proposed for the diagnosis of sepsis in the general ICU population. Recent advances in coagulation analysers have enabled the ability to obtain optical profiles of the clotting process for routine tests such as Prothrombin Time and APTT within 1 hour of the sample reaching the laboratory. The analysis using real time optical transmittance to provide more information from the clotting assay than just an endpoint clotting time. The profiles plot change in light transmittance against time, producing a 'wave-like' plot. Abnormal CWA have been seen in many conditions such as trauma and Disseminated intravascular coagulation (DIC) and especially in sepsis. Studies have shown that they predict for severe sepsis, septic shock and DIC [6,7]. Pilot studies in the general ICU have shown promise in the detection of severe sepsis[8]. Toh et al also found that this biphasic waveform was due to the rapid precipitation of CRP, and very-low-density lipoprotein (VLDL) in the presence of calcium ions. CRP belongs to a family of proteins known as pentraxins, and its level rapidly increases under conditions of infection or tissue damage[9]. Presence of abnormal waveforms has been confirmed in severe sepsis in studies performed in the general ICU[9] and in personal observations in University Hospital Aintree. It was identified that biphasic waveform was more accurate than PCT and CRP for differentiating patients with severe sepsis and septic shock, with 90% sensitivity and 92% negative predictive value[6,7]. These biphasic waveform values were significantly more abnormal during day 1–3 in septic nonsurvivors than in survivors and nonseptic nonsurvivors and exhibited the best specificity (91%) and negative predictive value (98%) for the prognosis of sepsis-related mortality on day 3[6]. There are no studies have examined the diagnostic value of these novel sepsis measures in the neurosurgical population of subarachnoid hemorrhage, traumatic brain injury and other intracranial hemorrhages.

Routine analysis of WBC using flow cytometery-based method has been shown to be low cost, rapid and can be incorporated as part of the routine full blood count (FBC) analysis. During stress or infection, less mature neutrophil forms enter circulation, including an increased number of bands. This is referred to as a left shift, which is defined as an elevated immature/total granulocyte ratio or an

elevated neutrophil band count. Neutrophil and monocyte activation has been shown to correlate well in laboratory studies and in pilot clinical studies on septic ICU patients[10]. Linssen et al showed that using the Sysmex XE-2100 analyser allowed the real time study of monocyte and neutrophil activation by using the routine fluorescence-flow cytometer of the analyser. The information about cell shape, cell membrane content and cell granularity, thus giving an idea as to WBC activation status. The 'Neutrophil X' (NEUT-X) parameter measures neutrophil granularity. The more the cells degranulate (in response to sepsis), the lower the parameter value becomes. The 'Neutrophil Y' (NEUT-Y) parameter measures the production and release of proteins (reactive oxygen intermediates and cytokines) from neutrophils by a change in nucleic acid content. The immature granulocyte parameter (IG) parameter and neutrophil reactive index (NEUT-RI) also correlates with sepsis. Wiemann et al (2015) has used a composite score, Intensive care Infection Score (ICIS) in medical-surgical ICU patients to differentiate between with infection and without infection with high sensitivity and specificity[11]. The ICIS comprised of five blood-cell derived parameters that characterize the early innate immune response: (I) mean fluorescence intensity of mature (segmented) neutrophils; (sNGI) (II) difference in haemoglobin concentration between newly formed and mature red blood cells;(dCHC) or delta-Hemoglobin (III) total segmented neutrophil count;(sN#) (IV) antibody secreting lymphocyte count;(ASL) (V) immature granulocyte count(aIG). In the study, an ICIS score of more than 3 was considered as positive score for sepsis using a modified fully automated routine fluorescence hematology analyser (XN-10; Sysmex, Kobe, Japan). This can be measured during the WBC measurement and does not require any additional blood sampling.

2. Study Aims and Significance

Primary Aim

To validate the use of APTT CWA and ICIS, as early sepsis markers for neurosurgical ICU patients suffering subarachnoid haemorrhage, traumatic brain injury and other intracranial haemorrhages.

Secondary Aim

To examine the evolution of CWA, immuno-parameters (KL-6, SP-A, MIG, presepsin) and various WBC activation markers over the time in relation to diagnosis of sepsis, development of positive blood cultures and mortality or recovery. Blood parameter measurements using a 3-part and 5-part differential analyser will be performed.

Potential Risks

This will purely be an observational study of changes in laboratory parameters and the research participants do not deviate from the current standard of care. However minimal risks are anticipated for this study as all additional blood samples collected are from arterial lines inserted as part of routine care for patients in the SICU.

Potential Benefits

There is no assurance that the research participants will benefit from participation in this study. However, their participation in this study may add to the medical knowledge by exploring novel early predictors and validation of laboratory parameters in the management of sepsis. This will greatly complement current clinical monitoring of patients in the SICU without subjecting the patients to additional invasive tests.

Study Significance

The research study is to explore novel early predictors and validation of laboratory parameters in the management of sepsis. This will greatly complement current clinical monitoring of patients in the SICU without subjecting the patients to additional invasive tests.

3. Study Design

This is a prospective observational study. 150 subjects with the admission diagnosis of neurological haemorrhage (e.g. subarachnoid haemorrhage, intracerebral haemorrhage etc), admitted to SICU of National University Hospital, Singapore, who are expected to stay for more than 48 hours, will be recruited and enrolled. The proposed workflow for this study is highlighted in Figure 1. No adverse events are expected as a result of this study as patients do not deviate from the current standard of care. Frequency of blood sampling will be stipulated at day 1/2/3/4/5 to draw clinical relevance. An additional 0.5 tablespoonful (7.7ml) of blood will be taken daily from each subject as well as residual blood from routine laboratory test blood samples. In total, an additional volume of 2.5 tablespoonful (38.5ml) of blood will be collected from each subject over a period of 5 days. Residual blood refers to any leftover blood samples collected for routine laboratory tests which will be shared and used to carry out additional blood tests as shown in Table 1. All ICU patients will have arterial lines inserted as part of routine care, so there will not be pain from the blood sampling. In the current study, measurements will be made on the routine laboratory haematology, coagulation and immunoassay analyzers. Research blood parameters that are not usually reported as indicated in the background section will be used for the correlation and association analysis to sepsis. Over a period of 2 years, these blood samples will be collected from 150 patients, stored in -80 degrees' freezer and then used for batch standard laboratory tests. The remaining blood samples will be stored in NUH in -80 degrees' freezer over a period of 5 years for future biomedical research use.

In addition, the study team will also be collecting clinical and demographic data of recruited subjects from medical records for purposes of this study.

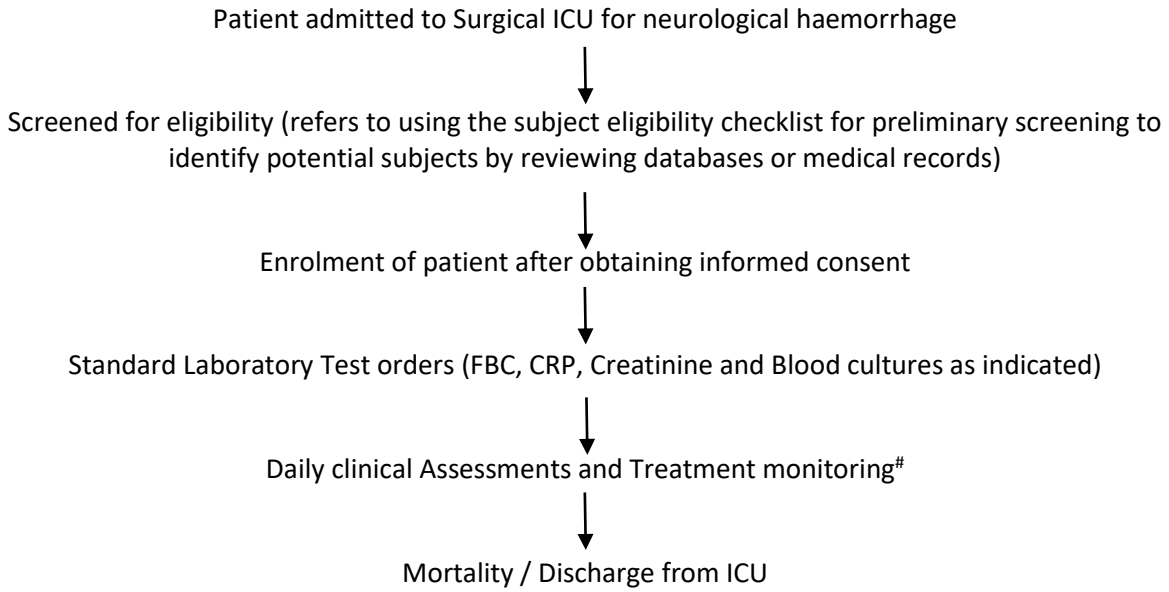

Figure 1. Proposed flow chart for research project

#The diagnosis of sepsis is obtained from the treating physician or the commencement of antibiotics and confirmed by microbiological cultures (routinely performed during this period as the standard of care to detect sepsis). The treating physician will not be involved in data analysis of CWA, immuno-parameters and WBC infection score. Results will be stratified based on patients who have no SIRS, SIRS but no microbiological evidence of sepsis (via blood, sputum, urine cultures etc), sepsis (SIRS + microbiological evidence) and retrospectively analysed. The patient will be treated according to the treating physician's routine standard of care practices in the best interest of the patient. No potential adverse events are expected as this will purely be an observational study of changes in laboratory parameters.

Source of sepsis secondary to ventilator associated pneumonia is defined according to the new CDC guideline for definition which includes

- Definition of ventilator associated event plus, on or after calendar day 3 of mechanical ventilation and within 2 calendar days before or after the onset of worsening oxygenation, following criteria to be met
- Criteria one: Positive culture of one of the specimens meeting quantitative or semi quantitative thresholds (Endotracheal aspirate $>10^5$CFU/ml; Bronchoalveolar lavage $>10^4$CFU/ml; Lung tissue $>10^4$ CFU/ml and protected specimen brush $>10^3$ CFU/ml without need for purulent secretions
- Criteria two: Purulent respiratory secretions plus organism identified from one of the above specimens without sufficient growth to meet criterion 1
- Definition of bacteraemia is by presence of positive blood cultures
- Catheter associated Urinary tract infection is defined as presence of positive urinary microscopic examination of more than 5 WBC from the freshly placed urinary catheter long with positive urinary cultures

Table 1. Blood volume required for investigation

Blood volume collection and testing:					
#Standard of Care Blood Investigations	DAY 1	DAY 2	DAY 3	DAY4	DAY 5
FBC (EDTA/Lavendar top)	3ml*	3ml*	3ml*	3ml*	3ml*
CRP (SSTII/Red Top)	5ml**	-	5ml**	-	5ml**
Blood cultures (routine ICU care for fever)	10ml	-	10ml	-	10ml
CREATININE (SSTII/Red Top)	5ml**	5ml**	5ml**	5ml**	5ml**
Additional blood investigations:					
FBC (3-Part Diff) (EDTA /Lavendar Top)	3ml*	3ml*	3ml*	3ml*	3ml*
PTINR/APTT (Citrate/Blue Top)	2.7ml	2.7ml	2.7ml	2.7ml	2.7ml
PROCALCITONIN (SSTII/Red Top)	5ml$	-	5ml$	-	5ml$
Serological/Inflammatory Markers (KL-6, SPA, MIG, Presepsis)(SSTII/Red Top)	5ml$	5ml$	5ml$	5ml$	5ml$

Actual blood investigation may vary depends on patient's clinical conditions
Volume marked with *, **, $ indicate sharing of blood tube for testing.

Note:

- 7.7ml of additional blood (highlighted in yellow) is taken for this study daily over 5 days for these additional blood tests: APTT, Procalctitonin and Serological/Inflammatory markers (KL-6, SPA, MIG, Presepsis). All other bloods are routinely taken for routine investigations during their SICU stay.
- Out of the 7.7ml of additional blood, 5ml of blood drawn will be shared and used for these blood tests: Procalcitonin and Serological/Inflammatory markers (KL-6, SPA, MIG, Presepsis) as marked with this symbol $.

4. Patient Recruitment

List the number of subjects to be enrolled

A total target number of 150 subjects with the admission diagnosis of neurological haemorrhage (e.g. subarachnoid haemorrhage, intracerebral haemorrhage etc), admitted to SICU of National University Hospital, Singapore, who are expected to stay for more than 48 hours, will be recruited and enrolled.

Criteria for recruitment

The SICU anaesthetist on duty would make first contact with potential participants of the research study. After obtaining consent, the SICU anaesthetic team will then make the necessary referral to the study team members who will then screen for their eligibility based on the subject eligibility checklist. These potential participants will be recruited by face-to-face contact via their legally acceptable representative when they are in SICU.

Inclusion criteria for subjects:

- Adults 21 years and above
- Clinical/radiological suspicion or confirmation of neurological haemorrhage

Exclusion criteria for subjects:

- Age below 21 years
- Prisoners
- Known pregnancy
- Do-not-attempt resuscitation status
- Requirement for immediate surgery
- Active chemotherapy/neutropenia (Neutrophil count $<1.0 \times 10^9$/L)
- Immuno-compromised
- Haematological malignancy
- Treating physician deems aggressive care unsuitable
- Unable to provide informed consent or comply with study requirements

Patient withdrawal

Patients will be allowed to withdraw anytime during the study without comprising their care.

Subject Replacement

Subjects who drop out will not be replaced to reach the minimum target number.

5. Data Collection

Data will be collected for all tests performed (raw data format and instrument files) and the clinical data includes

- Past medical history and demographic data
- Presence of SIRS; timing of sepsis and septic shock
- APACHE II, SOFA and other disease severity scoring systems
- Routine blood tests such as CRP and/or PCT
- Timing of Blood cultures and therapies (Inotropes and antibiotics etc.)
- Longitudinal test results to include PT/aPTT CWA, FBC, WBC infection scores including ICIS and related parameters (marked as ICU day 1 to day 5), immuno-parameters (KL-6, SP-A, MIG, Presepsin)
- Complications
- Length of ICU stay and mortality

6. Study Outcomes

The primary analysis of the study is to examine a number of laboratory test parameters and its association to sepsis as the clinical outcome. The associations between the presence or absence of the biphasic wave form in CWA, WBC activation parameters and clinical relevance of immuno-parameters at baseline and subsequent diagnosis of sepsis.

Secondary analysis will examine the potential predictive value of evolution of CWA and WBC parameters over time in relation to the diagnosis of sepsis, development of positive blood cultures and mortality.

7. Data Analysis

Data Quality Assurance

The study team will ensure that the data obtained from this research is accurate, complete and reliable.

Data entry and Storage

The data will be entered into excel sheet, and then be stored and handled.

8. Sample size and Statistical methods

Determination of sample size

This is a pilot study to determine the resources needed for a full scale study, thus 150 has been selected as the recruitment target.

Statistical and Analytical Plans

In order to assess the ability of baseline CWA, immuno-parameters and ICIS to discriminate between septic and non-septic patients, a receiver operator characteristic (ROC) curve will be constructed for each measure against presence of sepsis as determined by the treating physician (SIRS), and by microbiological culture of bacteria (severe sepsis). As a result, the sample size is based on that needed to evaluate the area under the ROC curve. We assume that an AUC of 0.7 (while better than chance) would be insufficiently discriminating, but as one aim of the study is to estimate the AUC, a value of 0.85 has been set as the value expected. If this is the true value, then the sample size to rule out a value of 0.7 or less, with 80% power and one-sided significance of 5% is 98 patients, assuming the proportion of patients with sepsis is at least 25%.
Simple descriptive statistics (proportions and accompanying confidence intervals) will be used to assess complication rates and other frequency data. Continuous measures will be assessed using mean and standard deviation (or median and interquartile range if the distribution is non-symmetrical). A number of further exploratory analyses may then be undertaken. Simple line plots of CWA and WBC activation parameters for each patient over time and summary measures or relevant repeated measures regression techniques may be considered. If feasible this may include the analysis of the relationship between sepsis confirmation and measures of CWA, immuno-parameters and ICIS over time. Time to event analyses may also be undertaken using standard techniques (e.g. Kaplan-Meier plots, Cox proportional hazards models) to compare the survival prospects and/or time to sepsis for patients with and without SIRS or severe sepsis at baseline.

9. Ethical Considerations

Informed consent

Informed consent will be obtained prior to performing any study-related procedures. The informed consent process will be taken at SICU via the potential participants' legally acceptable representative (LAR). The LAR would be given sufficient time to decide whether to let them participate in the research study. The consent will be conducted in a private room in SICU together with participants' LAR. The wishes of the participant's LAR must also be respected if they chose not to allow the participant to take part in the research study.

IRB review

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by the IRB/ NHG DSRB/ SGH CIRB.

Confidentiality of Data and Patient Records

For hardcopy data, they will be stored in designated locked cabinet(s) or room(s) that are accessible to authorized study personnel only.

For electronic data, they will be stored on in a secured computer that is password protected. The databases will not contain subject identifiers and the data linking subject identifiers and the subject identification codes will be stored separately.

10. Retention of study documents

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation should be retained by the PI in a secure storage facility for 6 years or minimum duration of retention period as specified by the institutional policy. The records should be accessible for inspection and copying by authorized authorities.

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