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Level of Protein C Activity as a predictor of mortality in pediatric ICU patients with severe sepsis.

Study Purpose and Rationale:
Sepsis is a leading cause of mortality in pediatric Intensive Care Units (ICUs) in the United States. Each year over 43,000 children are diagnosed, of whom approximately 10% die from sepsis and sepsis-related causes (1,2). Severe sepsis is the 4th leading cause of death in the United States for children under 1 year of age and is the 2nd leading cause of death in children ages 1-14 years with an estimated cost to the health care system of $1.97 billion annually (1,2).

Over the past 10 years a significant amount of research has been done in the adult population to determine accurate outcome predictors as well as new early goal-directed therapies in patients with sepsis. In 2008 the Society of Critical Care and the European Society of Critical Care Medicine published *International Guidelines for the Management for Severe Sepsis and Septic Shock* as part of their Surviving Sepsis Campaign (3). These recommendations provided evidence-based recommendations for both adults and pediatrics on the best care currently available for patients with severe sepsis. Included in this is the recommendation to administer recombinant activated protein C (drotrecogin alpha) in adult patients with septic shock or who are determined to be at high risk for death. Currently there is no recommendation for activated protein C administration in pediatrics and there remains controversy over whether or not it would be beneficial.

The rationale behind this recommendation in adults comes from the understanding of the interplay between the inflammatory cascade and the development of a procoagulant state. Inflammatory cytokines produced during infection stimulate the coagulation cascade and inhibit fibrinolysis, and in turn the coagulation/fibrinolytic pathways stimulate inflammation creating a prothrombotic cycle. (4) This dysregulation can result in microvascular thrombi, organ dysfunction and even disseminated intravascular coagulation (DIC).

Protein C, when activated, is an important anticoagulant and profibrinolytic enzyme that is able to inactive coagulation factors Va, VIIIa and plasminogen activator inhibitor 1. However, when certain inflammatory cytokines like TNF are present, they decrease the amount of thrombomodulin, a protein that is crucial in the activation of protein C. Without thrombomodulin protein C cannot function and the antithrombotic effects are lost. This finding led to the observation in adults that noted increased risk of death with lower levels of activated protein C (aPC). This observation led to several clinical trials studying the effects of administering recombinant activated protein C in adults with sepsis with mixed results.

The evidence showing a benefit to administering recombinant activated protein C in pediatrics is limited. Only three trials have been completed in pediatric patients. The first was a Phase 1B, FDA-mandated, open-label, non-randomized trial showed possible benefit

to giving recombinant activated protein C to septic children. (5) A follow-up, open-label study (ENHANCE trial) on administering recombinant aPC was done to obtain further disease state and safety information in both adults and children. They did find a significant association between low levels of aPC after infusion and increased risk of mortality, however lack of a placebo group limited conclusions that could be drawn. (6) A third study started in children (RESOLVE trial) was stopped prematurely after it showed no benefit to replacement of activated protein C, and in fact raised the question of safety in light of higher numbers of intracranial hemorrhage in the treatment arm (though this difference was not statistically significant). (7)

In light of these conflicting studies, the current recommendations do not recommend giving activated recombinant protein C to pediatric patients, but more study on this topic needs to be done. The first step in this process is evaluating protein C activity in children. We know that healthy neonates have a lower activity level than older children and adults and that septic children have overall lower protein C activity than their healthy counterparts, with protein C deficiency defined as levels <80% of normal, and severe deficiency defined as <40% of normal. However, to date there have not been consistent data showing that protein C activity level in septic patients correlates with death.

As part of the ENHANCE study, protein C activity in children was evaluated. The study's purpose was to determine whether or not administering recombinant activated protein C to adults and children resulted in reduced risk of mortality. They also looked at the differences between protein C activity levels in survivors of sepsis compared to non-survivors and found no statistical difference between survivors (42.3%) vs. non-survivors (35.5%). The study was underpowered, however, due to limited number of blood samples available for evaluation. (6) However a study done by Samransamruajkit et al in Asia in 2007 showed a statistically significant difference in aPC levels in non-survivors of septic shock vs. survivors (23.6% vs. 46.8%, $p = 0.002$). (8)

If those children who die from sepsis are shown to have a significantly lower level of protein C activity compared to children who survive sepsis then we might not only be able to use protein C as a marker for mortality but we might also be able to study whether targeted therapies such as activated recombinant protein C in certain subsets of patients at higher risk for mortality based on protein C activity would improve survival.

In light of these conflicting results and in an effort to determine a better predictor of mortality in septic pediatric patients, this study aims to determine whether aPC level is associated with mortality in patients with sepsis or septic shock.

Study Design and Statistical Analysis:
This is a prospective minimal risk study that will enroll 250 patients with severe sepsis assuming an approximate mortality rate of 10%. This will allow us to detect a difference in activated protein C activity of 20 percentage points between survivors and non-survivors, and achieve 80% power using an unpaired T-test with alpha = 0.05 and assuming a survivor : nonsurvivor ratio of 9:1. We also plan to do an exploratory secondary analysis

using multivariable logistic regression to estimate associated risk factors of mortality, including child demographics, co-morbidities, and clinical data described below.

Study Procedure
Patients between the ages of 1 month and 18 years who are diagnosed with severe sepsis or septic shock (as defined below) will be eligible. Patients will be recruited from the pediatric ICU's of four medical centers from December 1, 2010 through November 30, 2012. Written informed consent from parents/legal guardians will be obtained. Data will be collected on the patients for a total of 28 days from admission to the PICU and will include mortality, vital signs, lab values and ICU length of stay. Within the first 24 hours of admission to the PICU we will obtain 5mL of blood at a time when the patient would already be having blood drawn for clinical purposes. The blood will be collected in a sodium citrate tube and stored in the core laboratory at -70 degrees with anonymous identifiers. The tubes will then be sent to an outside laboratory for chromogenic analysis of activated protein C levels.

Primary Outcome:
All-cause Mortality

Secondary Outcome:
Length of ICU stay, composite time to complete organ failure resolution (CTCOFR)

Variables to be Collected:
1. Demographic information
    a. Age
    b. Gender
2. Co-morbidities
    a. Immune status
    b. Congenital heart disease
    c. Congenital/acquired hematologic disorders
3. ICU data
    a. Primary and final diagnoses
    b. PRSIM III score
    c. Vital signs on a daily basis
4. Laboratory data (CBC, BUN, creatinine, culture results) on a daily basis
5. Medications
    a. Antibiotics (type, duration, total number)
6. Pressors/vasoactive drugs (type, dose, number and length of use)
7. Length of stay
8. Final outcome (i.e, mortality vs. discharge to floor vs. other)

Study Drugs:
None

Medical Device:
None

Study Questionnaires:
None

Study subjects:
Inclusion Criteria:
Patients ages 1 month – 18 years admitted to the pediatric ICU with a diagnosis of severe sepsis as defined by:

- Evidence of infection (either by culture or suspected by investigator)
- Must meet 2 out of 4 SIRS criteria (9)
    - Temperature greater than 100.4 or temperature instability taken using rectal, central or oral methods
    - Heart rate > 90^{th} percentile for age
    - Respiratory rate > 90^{th} percentile for age or new need for mechanical ventilation (for example, cannot be ventilated on a regular basis at home due to underlying condition)
    - WBC count >12,000 or < 4,000 (not secondary to chemotherapeutic agents or chronic steroid use) OR if immature neutrophils > 10%
- Must have $\geq$ 1 organ system dysfunction (renal, cardiovascular, respiratory, hematologic or unexplained metabolic acidosis) that occurred within 48 hours of enrollment.

Exclusion Criteria:
Diagnosis on admission to PICU of end-stage organ disease
Known history of protein C deficiency
Receipt of blood products within previous 3 months

Recruitment of Subjects:
A study coordinator will review admissions to the ICU daily and determine eligibility of the patients via review of the electronic and paper medical record. If eligibility criteria are unclear from this preliminary evaluation the study coordinator will approach the parent of the admitted child (or legal guardian) after seeking permission from the medical team. If granted permission, the study coordinator will then approach those patients meeting inclusion criteria and lacking exclusion criteria to obtain written informed consent from the parent or legal guardian.

Confidentiality of Study Data:
All written data regarding study participants will be kept in a locked room and all electronic data will be kept on a secure computer in a password-protected file. Blood samples will be collected in similar tubes and each tube labeled with a non-identifying number. Only study personnel will have access to the file linking study sample number with patient name and medical record number.

Potential Conflict of Interest:
None

Location of Study
Morgan Stanley Children's Hospital of New York-Presbyterian pediatric ICU as well as 3 other sites to be determined.

Potential Risks:
There are no foreseeable risks to any of the children or families enrolled in the study.

Potential Benefits:
Patients enrolled in the study will not benefit however this may be important in the care of future patients.

Alternative Therapies:
Not applicable

Compensation:
None

Costs to the Subjects:
There is no monetary cost to any patient or family enrolled in the study. The patient's family will be asked to provide written informed consent for a one-time blood draw of 5 milliliters of blood from their child when blood is already being drawn for clinical purposes.

Minors as Research Subjects:
To be submitted to the Department of Pediatrics Committee on Human Investigation

Radiation or Radioactive Substances:
None

References

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5. Barton P et al. Safety, pharmacokinetics, and pharmacodynamics of drotrecogin alpha (activated) in children with severe sepsis. *Pediatrics*. 2004; 113:7-17.

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8. Samransamruajkit R et al. Levels of protein C activity and clinical factors in early phase of pediatric septic shock may be associated with the risk of death. *Shock.* 2007; 28(5) 518-23.

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