B-Type Natriuretic Peptide For Early Detection of LPS-Induced Cardiac Dysfunction

Abstract

Sepsis remains the leading cause of death in the intensive care setting. Due to the complex systemic inflammatory cascade seen in these patients, multiple organ systems may become dysfunctional if the infection persists. The development of marked cardiac dysfunction is associated with a poor prognosis. Lipopolysaccharide (LPS) is a key bacterial substance that has been shown to mediate the hemodynamic changes and organ system dysfunction seen in sepsis. This study is focused on exploring the use of B-type Natriuretic Peptide (BNP) blood levels as a biological marker for the presence of LV dysfunction in septic patients and in animals treated with LPS. BNP may serve as an early and accurate diagnostic tool in the management of septic patients because it is produced predominantly by the left ventricle in response to ventricular dilatation. LV dilatation as well as systolic dysfunction (decreased ejection fraction) have been shown to occur in septic patients as well as animals and humans treated with LPS. The ability to identify the presence of cardiac dysfunction in a timely manner using BNP levels would lead to earlier diagnosis and changes in the treatment plans for these patients. In this project, patients from the VA Medical Center that have been diagnosed with sepsis will have their BNP levels monitored on a serial basis. If their BNP levels rise to a level indicative of cardiac dysfunction, an echocardiogram will be performed in order to document changes in cardiac dimensions and function. Similarly, the proposed animal model will attempt to show a rise in plasma BNP that correlates with LPS induced ventricular dysfunction as determined by echocardiography at various time intervals. Analysis of data between the two models may reveal parallel findings in BNP levels and cardiac dysfunction. This would further implicate LPS as the molecular catalyst of sepsis related cardiac dysfunction.

Background

From 1979-1987 there were substantial increases in the occurrence of sepsis in the U.S., which was reflected in its rank as the 13th leading cause of death in the general population. In 1990, sepsis was reported as the most common cause of death in noncardiac intensive care units. The most recent data available from the Centers for Disease Control and Prevention ranks sepsis as the 12th leading cause of death in the general population. Despite extensive research and new supportive treatments, sepsis has persisted as a leading cause of death.

The course of treatment for septic patients currently consists of administration of antimicrobial agents appropriate for the infecting microorganism. The efficacy of this treatment is improved when it is instituted early in the course of the patient’s sepsis. However, this early treatment does not always occur for various reasons. One such reason is that septic patients often do not have positive blood cultures and even if they do there is a delay in obtaining the results of these cultures. There may also be delays in clinical recognition and diagnosis of sepsis. One study found positive blood cultures in only 17% of patients with sepsis, 25% with severe sepsis, and 69% with septic shock. The complex pathogenetic mechanisms and inflammatory cascade of sepsis can cause progression to organ dysfunction, hypoperfusion, and
hypotension. At this point of severe sepsis, patients are admitted to an ICU. Today, patients who have progressed to septic shock have a 50% survival rate once admitted to the ICU (before ICUs existed more than 90% died).  

Several large-scale multi-center clinical trials have failed to reduce mortality from sepsis. Most of these studies involved non-glucocorticoid anti-inflammatory agents. The number of deaths due to sepsis' progression might be reduced if physicians could monitor a marker for the initial onset of organ dysfunction. Cardiac dysfunction is one of the primary physiological indicators of advancing sepsis. Septic patients display clear myocardial depression and other cardiovascular dysfunction. The continuum from sepsis to septic shock is marked by increasing organ dysfunction accompanied by increased mortality. Consequently, a biological marker to indicate the progress of such dysfunction would be an indispensable diagnostic tool.

The prognosis of patients with sepsis might be improved if cardiac dysfunction is recognized to allow therapeutic interventions at its onset. B-type natriuretic peptide (BNP) is a biological marker of cardiac dysfunction (dilatation), especially in the left ventricle where it is predominantly produced. Early detection of cardiac dysfunction through BNP levels may signal the progression or presence of sepsis. This would allow for aggressive medical treatment to prevent organ dysfunction. For example, septic patients with cardiac dysfunction, as demonstrated by elevated BNP levels, would receive inotropic drugs rather than more fluids. With earlier detection of progression and therefore more focused treatments, deaths due to this century-spanning syndrome might finally be decreased.

**Definition**

BNP has been shown to be a strong indicator of cardiac dysfunction in patients who are admitted to the hospital with symptoms of heart failure. BNP is produced almost exclusively in the left ventricle of the heart in response to dilatation of the chamber and/or increases in LV filling pressure. BNP is both rapidly produced and turned over and thus is a marker that accurately predicts present conditions. Plasma BNP levels are normally about 30-45 pg/ml. When these levels increase to above 100 pg/ml there is a strong positive correlation with the presence of cardiac dysfunction in congestive heart failure patients. Left ventricular dilatation is not only a prominent feature in sepsis but also an early response to lipopolysaccharide (LPS) in normal volunteers. Thus it is anticipated that plasma BNP will be an accurate marker of cardiac dysfunction and dilatation in septic patients. It is hypothesized that elevations in BNP above 100 pg/ml will serve as a useful biological marker for detecting dilatation associated with cardiac dysfunction in the hearts of septic patients.

The goal of this project is to show that plasma BNP levels can be used to diagnose ventricular dysfunction in patients with sepsis. In the clinical setting of sepsis, it is very difficult to diagnose ventricular dysfunction. Patients are very ill, and most attention is paid to giving patients antibiotics, fluid and medication to support the blood pressure. By the time CHF is discovered, it is often too late to help the patient. Thus, early detection of LV dysfunction should be extremely valuable in this setting. The use of BNP levels to make the diagnosis of sepsis associated CHF (cardiac dysfunction) is a new and innovative approach.
This project is a combination of current research interests of all three ISP committee members. Dr. Lew is currently involved in exploring the effects of LPS on cardiac myocytes and the apoptosis cascade that results. Dr. Maisel is researching the utility of BNP in various clinical settings such as dyspnea associated CHF. Dr. Roth is currently investigating heart failure in animal models. The student will perform the animal protocol in its entirety (see Methods) while receiving instruction regarding the use of the echocardiography machine. The student will be responsible for generating measurements of cardiac dimensions in both control and experimental animals from echocardiographic studies. The student will analyze the results of these echocardiography studies and present his findings at weekly data meetings with the ISP Chair, Dr. Lew. The animal protocol will be carried out in Dr. Lew’s laboratory on the 6th floor of the VA Medical Center. In addition to the animal protocol, the student will work to identify, with the help of the committee, patients with sepsis to enroll in the study. The student will obtain informed consent from such patients and then serve as the main coordinator responsible for seeing the patient all the way through the protocol. This would include serial monitoring of plasma BNP levels and coordinating echocardiograms for patients when appropriate. The student will collect all relevant data and maintain a database for further analysis during the course of the study. The proposed work will be carried out during 2½ months of the 2001 summer (full-time) as well as elective time in the second year of medical school. The ISP project will be continued and finalized during 2-3 months of ISP time in the fourth year of medical school.

Methods

An animal model developed by Dr. Lew will be used in order to accurately correlate increases in plasma BNP with changes in cardiac dimensions and function. Rats will be administered controlled amounts of LPS that will cause ventricular dilatation with or without sepsis like cardiovascular conditions. LPS is the primary bacterial component responsible for initiating a systemic inflammatory cascade in many septic patients and those with sepsis like symptoms.4,8 Blood will be drawn on the animals in order to monitor BNP levels in response to LPS at various time intervals. Echocardiography will be used to confirm and measure the cardiac dilatation that occurs in response to the LPS.

The rats will be anesthetized with isoflurane for all blood draws and echocardiography studies. Rats in two different experimental groups will be administered 1mg/kg or 5mg/kg of LPS that will generate sepsis like cardiovascular conditions, especially ventricular dilatation. BNP levels will be monitored using a radioimmunoassay at 24 and 48 hours after administration of LPS. Echocardiography will be used to confirm and measure the cardiac dilatation that occurs in response to the LPS. Echocardiographic measurements will be made initially, before the LPS injection, and at 1, 6, 24, and 48 hours post injection. A control group will receive intravenous saline injections. All rats will receive fluid replacement, 1.5 ml of saline, subcutaneously after the 24 hour blood draw. Echocardiography measurements as well as BNP levels will also be recorded for the control group.

The criteria for patient inclusion into the clinical study will be two fold. First, patients with positive blood cultures will automatically qualify as patients with bacteremia. Second, in order to identify patients at risk for developing sepsis, patients who meet the following criteria
for infection and/or systemic inflammatory response syndrome (SIRS) will be enrolled. Patients must have one or more of the following to meet the infection criteria: white cells in a normally sterile body fluid; perforated viscus; radiographic evidence of pneumonia with the production of sputum; a condition associated with a high risk of infection. Patients may also be included if they meet three of the following SIRS criteria: a core temperature of ≥38°C (100.4°F) or ≤36°C (96.8°F); a heart rate of ≥90 beats/min; a respiratory rate of ≥20 breaths/min or a PaCO₂ of ≤32 mmHg or the use of mechanical ventilation for an acute respiratory process; a white cell count of ≥12,000/mm³ or ≤4,000/mm³ or a differential count showing ≥10% immature neutrophils. All patients will be taken from the VA Medical Center patient population and will be over 18 years of age.

The symptoms of the above-defined SIRS can present in patients due to noninfectious syndromes (not sepsis) such as surgery, trauma, pancreatitis, erythroderma, and transplant rejection. For the purposes of this study the patients that present with any of these conditions and are lacking evidence of endotoxemia (LPS in blood), may serve as useful negative controls.

Plasma BNP levels will be recorded serially, every 1-3 days, on consenting patients who meet the above stated inclusion criteria. BNP will be measured using the Triage B-Type Natriuretic Peptide test (Biosite Diagnostics Inc., San Diego, California). The Triage BNP test is a fluorescence immunoassay for determining BNP concentration in whole blood or plasma. The symptoms and treatment of the patients will be recorded. Also, the results of blood cultures and/or any clinical diagnosis will be noted. If a patient’s BNP level exceeds 100 pg/ml an echocardiogram will be performed in order to assess cardiac dimensions (especially left ventricular dilation) and the presence of cardiac dysfunction. Such a patient will continue to have blood drawn serially and tested for BNP concentration. After the patient’s BNP level drops back down below the 100 pg/ml mark, another echocardiogram will be performed to assess changes in cardiac dimensions and function. Patients with a known previous history of congestive heart failure at the time of admission will have a baseline BNP drawn. If BNP levels increase to above 50% of the baseline, an echocardiogram will be performed. A late recovery endpoint for all subjects will also be recorded.

**Evaluation**

This project will be evaluated on a consistent basis at weekly data meetings (during the summer months). The ISP committee members will provide final evaluation of the student’s data collection and analysis for both the animal model and clinical study. The student will also be evaluated on the final analysis and conclusions with regard to correlating the two models. This final analysis will be in the form of appropriate spreadsheets, graphs, and a written description of the outcomes. Success of this project will be determined by attainment of new insight into BNP’s effectiveness in detecting cardiac dysfunction in septic patients.
References


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