

Spring 2017

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Recommended Citation

Roberts, Jose M., "Biomarkers of Sepsis: A Retrospective Approach" (2017). *Honors Research Projects*. 534.
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Biomarkers of Sepsis: A Retrospective Approach

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May 2017

Keywords: biomarkers, sepsis, hospital admission, hyponatremia, fever, c-reactive protein

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Abstract

Background: Biomarkers are taking the spotlight in becoming the norm for early diagnoses. Sepsis is an inflammatory disease that increases metabolic rate in children. The first biomarker is hyponatremia. Hyponatremia is a frequent electrolyte imbalance in clinical practice, often observed in children with inflammatory disease and infection. Presence of hyponatremia is associated with electrical signaling imbalances, inflammation and renal dysfunction. The clinical value of hyponatremia in pediatric patients is unknown. The C-reactive protein is a second biomarker. Its presence signifies that necrotic cells and inflammation are present.

Objectives: To evaluate the use of biomarkers in children seen in the Emergency Department with a diagnoses of sepsis.

Design/Methods: This is a retrospective study including children between 11 months -18 years old presenting to Akron Children's Hospital ED with fever and sepsis between January - December 2014. Electrolytes were collected in 731 patients, and 31 patients were excluded due to immune insufficiency or diuretic use. Hyponatremia was defined as serum sodium levels of ≤ 132 mEq/l, and patients were divided into two groups accordingly. Outcomes of interest included hospital admission and length of hospital stay (LOS). Categorical variables were presented as counts and percentages and were compared using χ^2 . The Wilcoxon Rank Sum test was applied to compare non-normally distributed variables between groups. Spearman correlations were evaluated between continuous variables.

Results: Twelve percent ($n = 84$ vs. $n = 616$) of patients had hyponatremia. Patients with hyponatremia had higher serum CRP and lower serum bicarbonate levels. Hyponatremia was associated with a higher admission rate and longer LOS (days) (see Table 1). Spearman correlations coefficients revealed correlations between hyponatremia, maximum temperature in ED ($r_s = -0.17$, $P < 0.0001$), bicarbonate ($r_s = 0.13$, $P = 0.0008$), white blood cell count ($r_s = -0.08$, $P = 0.0449$), anion gap ($r_s = 0.13$, $P = 0.0005$), and CRP ($r_s = -0.23$, $P = 0.0004$). Patients with hyponatremia are 1.79 times ($P = 0.0211$, 95% CI 1.08 – 2.95) more likely to be admitted.

Conclusion: In febrile children with sepsis, hyponatremia was associated with higher levels of inflammatory marker CRP, elevated leukocytes, and acidosis. Patients with hyponatremia had higher admission rates and longer LOS. The correlation was low, but the use of hyponatremia and CRP as potential biomarkers in diagnosing sepsis early could be better understood in future studies.

Introduction

The study of life sciences is highly attributed to an ability to predict outcomes based upon logical deduction. In the case of pediatric care, sepsis remains the number one cause of death due to infection in the developed world, affecting more than six million newborns and children annually (World Sepsis Day, 2015). Sepsis is an inflammatory and bloodstream infectious process that develops at birth or a few hours after birth. Early onset occurs during delivery from the amnion or birth canal, while late onset may occur 72 hours after birth (Bentlin et al., 2010). Late onset may be attributed to bacteria, a virus, or a fungus from the neonatal area of the hospital or contamination from health care workers (Carl et al., 2014). Despite efforts for preventing sepsis through assessment of cleanliness, aseptic techniques, and hand hygiene, it still occurs at an alarming rate (Glasper, 2016). Moreover, according to Sepanski et al., (2014) “in the United States alone, there are an estimated 751,000 cases per year with an annual cost of \$17 billion. Between 20,000 and 40,000 US children develop septic shock annually (p.3).” Early recognition of sepsis symptoms may prevent undesirable outcomes.

If signs and symptoms are not recognized, full circulatory collapse and multiorgan failure causes mortality. Frequently, pediatricians fail to identify specific clues that point to the initial stages of sepsis. Blood biomarkers may prove beneficial in this area of study. The use of blood biomarkers from routine laboratory assays to aid in diagnoses and treatment of sepsis is well established (Moseley, 2004). A biomarker frequently seen in clinical practice is hyponatremia. Hyponatremia is defined as abnormally low sodium levels. Assessment of hyponatremia can easily be attained via a basic metabolic panel (BMP) (Goh, 2014). C-reactive protein (CRP) levels are also routine laboratory assays, and its evaluation allows physicians to assess systemic inflammation.

Background

Sepsis ensues when an infection yields a hyperactive immune response. The cellular pathways that govern inflammation are overexpressed leading to severe inflammation of systemic tissues (Sepanski et al., 2014). Fever, hyponatremia and an incidence of high CRP serum levels are easily accessible biomarkers that may help diagnose a sepsis infection early. Early diagnoses will allow for a timely response initiated by health care workers, preventing its transition into more severe stages.

Sepsis is a deleterious infection that increases in severity as it progresses into latter stages. Sepanski et al., (2014) mentioned that diagnoses of a septic shock development in children could be challenging because of the natural ability of children to compensate and mask signs and symptoms of sepsis in its early stages. According to Mayo Clinic (2016), physicians divide the infection into three distinct stages: sepsis, severe sepsis and lastly septic shock. Each stage has different signs and symptoms. Utilizing serum biomarkers allows physicians to recognize the infection early and act promptly. Guidelines to treat sepsis include sensitive interventions such as antibiotic therapy, administration of fluids and hospitalization of the child.

Identification of progression to latter stages is difficult to evaluate. An infection diagnosed as sepsis must have two of the following three symptoms; an increased basal body temperature that is classified as a fever, a heart rate of ninety beats or higher per minute, or a respiratory rate greater than twenty breaths per minute. Children will naturally have higher baseline values when compared to adults. Progression into severe sepsis is characterized by systemic changes, notably an increase in metabolic rate, dehydration and depletion of electrolytes, which can be assessed by a BMP (Goh, 2014). Symptoms at this stage may include a decrease in urinary output, difficulty breathing as well as abdominal pain and severe

inflammation (Carl et al., 2014). Finally, septic shock is inclusive of the latter symptoms, as well as hypotension. Low blood pressure causes inadequate perfusion of tissues, leading to ischemia and multi-organ failure. Mortality is still a present theme in both developed and underdeveloped countries (Riedel & Carroll, 2012). The ability to quickly and accurately diagnose sepsis is of great importance to future advances in medicine.

According to Kruger (2010), there is great potential in using several biomarkers as a way to predict pathogenic disease due to their reflectiveness of the metabolic pathways that are affected. First, diagnoses of one symptom of sepsis can easily be achieved with a simple temperature test. A clinically significant fever is characterized as one above 38°C (100.4°F) (Hamilton, 2013). The change in temperature may be induced by an antigen that the body recognizes as foreign eliciting a specific immune response, increasing B-cell proliferation and differentiation (Gleeson, 2007; Grey et al., 1961). Warmer temperatures assist in leukocyte activity, making extravasation to the infected tissue much faster as well as increasing enzymatic activity (Grey et al., 1961). In a localized infection, first responding cells and cells adjacent to the infection secrete signaling molecules. These molecules, termed cytokines and lymphokines, recruit more immune cells and cause localized inflammation (Hack, 1997). Hamilton (2013) also states that the epidemiology of fever in children has changed dramatically with the introduction of vaccines. Medical advances now allow children to be exposed to antigens of many different bacterial and viral strains inducing a protective feature that could last a lifetime (Hamilton, 2013). Evolution of pathogens occurs alongside our attempts, at times indirectly making pathogens more resistant to medical intervention.

Next, electrolyte imbalances may provide crucial clues to diagnose a sepsis infection, specifically hyponatremia. Hyponatremia is a frequent electrolyte abnormality seen in clinical

practice, and can be defined as a blood serum concentration of Na <135 mEq/L (Goh, 2004; Hasegawa, 2008). Hasegawa (2008) also explains that hyponatremia is observed in inflammatory diseases. A sepsis infection is both inflammatory and characteristic of neuroendocrine dysfunction (Santos, 2013). Sodium is important in establishing concentration gradients, maintaining resting membrane potential and excitability (Goh, 2004). Dehydration, a symptom of septic shock, is due to an increased metabolic rate which leads to hypotension (Sahay et al., 2014). Homeostatic mechanisms in the kidneys and heart will activate the Renin-Angiotensin-Aldosterone hormonal cascade to increase blood volume by promoting water and sodium reabsorption in the nephrons of the kidneys, ultimately increasing pressure (Santos, 2013; Sahay et al., 2014). Blood vessel dynamics are also affected with sodium loss. Vasoplegia, the impairment of vascular reactivity, affects endothelial cells and also contributes to the hypotension attributed with sepsis (Sharaway, 2014). Vasopressin also plays a role in the hormonal cascade. Its primary function is to promote water reabsorption, which would describe the decrease in urinary output. Water loss and electrolyte loss are directly related, and they both can be initiated by a fever.

Lastly, sepsis infections are characteristic of inflammation. Inflammation is a response of many cell signaling pathways. There is a limit on the synthesis of pro-inflammatory molecules but in the case of sepsis the mechanisms are not in sync (Pittman, 2011). One specific signaling molecule made in the liver, the C-reactive protein (CRP), is specialized to recognize foreign pathogens and damaged cells of the host. CRP works to tag unhealthy cells and initiate their elimination (Que, 2015). According to Torres et al. (2012), abnormal levels of CRP may be a significant biomarker in determining early onset of sepsis. As more tissues are damaged, more CRP is secreted leading to abnormal detectable levels via lab assay (Que, 2015). Inflammation

can be plainly termed as swelling of tissue, but it is important as it decreases the rate of exchange between foreign particles of the infection and the cells of the body thereby localizing the antigens. Vasodilation of vessels occur, even with incidences of vasoconstrictors in the blood (Giusti-Paiva, 2010). This mechanism may also act as a factor to systemic hypotension. High amounts of CRP targets a large population of healthy somatic cells which are then degraded by leukocytes. The cycle repeats, as more healthy cells are destroyed, more inflammatory markers are secreted. The key to stopping the positive feedback loop is still unclear.

Diagnosing sepsis in its early stages requires discerning essential symptoms from the overwhelming effects of the particular illness. Fever, hyponatremia, as well as incidences of increased serum CRP are most likely indicative of a sepsis infection. We hypothesize that these biomarkers may allow physicians to diagnose a sepsis infection and determine whether a sepsis infection is bacterial or viral. Additionally, these biomarkers may allow a physician to predict patient admission or discharge, as well as predict length of stay when they are admitted.

Methods

The retrospective study occurred at Akron Children's Hospital, the largest pediatric healthcare provider in northeast Ohio. With the help of the Rebecca D. Considine Research Department, the hospital charting program EPIC was used to collect data from children between 11 months -18 years of age presenting to the Akron Children's Hospital Emergency Department (ED). Criteria for selection included a fever and a final diagnoses of sepsis. Data was collected between the months of January-December 2014. BMPs were used to collect electrolyte information in 731 patients, and 31 patients were excluded due to immune insufficiency or diuretic use. Hyponatremia was defined as sodium blood serum concentrations of ≤ 132 mEq/L. C-reactive protein lab assays were also present in patient encounters.

Materials

For the research to take place, IRB approval was submitted. The patients admitted into the study were identified by an MRN number to ensure the protection of privacy for all Akron Children's patients. The patients were analyzed by looking through the EPIC charts and encounters to categorize each variable. Variables included vital signs such as respiratory rate, heart rate, blood pressure, and temperature. Laboratory assays included BMP, radiography, complete blood cell count with differential (ratio of white blood cells in the blood), CRP levels, and urinalysis to identify bacterial infections. These were the complete array of biomarkers used to predict early sepsis.

Data Analysis

Patients were divided into two categories based upon their BMP results: hyponatremia (≤ 132 mEq/L), or normal sodium levels (≥ 132 mEq/L). To analyze the data, a Wilcoxon Rank Sum test was applied to compare non-normally distributed variables between groups because the sample sizes of each group were greatly skewed ($n=84$ vs $n = 616$). Important outcomes included hospital admission and length of hospital stay (LOS). Categorical variables were presented as counts and percentages and were compared using χ^2 test. Spearman correlations were evaluated between continuous variables. Logistic regression models were developed to evaluate admissions based on potential predictors selected at the univariate analysis. Level of significance was set at 0.05.

Results

Twelve percent ($n = 84$ vs. $n = 616$) of patients were diagnosed as hyponatremic. There was a positive correlation between hyponatremia and serum CRP. Hyponatremia was associated

with higher admission rates and longer days of stay (See Table 1). Spearman correlations coefficients revealed correlations between hyponatremia and the following variables: maximum temperature in the ED ($r_s = -0.17, P < 0.0001$), Bicarb ($r_s = 0.13, P = 0.0008$), WBC ($r_s = -0.08, P = 0.0449$), and CRP ($r_s = -0.23, P = 0.0004$). Length of stay as well as the nature of the infection were not clearly identified.

Table 1: Shows results of the statistical analysis. Left side of the panel represents hyponatremic patients (n=84). Patients on average had higher CRP and LOS. Right side of the panel denotes normal sodium patients (n=616) and on average had lower CRP and shorter LOS.

Wilcoxon Rank Sum test									
	Present, n = 84 (12%)				Not Present, n = 616 (88%)				P
	n	Median	Min	Max	n	Median	Min	Max	
Age (yrs)	84	5	0.92	19	614	4	0.04	19	0.1559
CRP	33	9.3	0.4	43.13	190	4.36	0.2	39.4	0.019
Neut/Lymph Ratio	76	3.15	0	91	502	3.73	0	87	0.6418
Band	72	15	0	58	460	12	0	84	0.0921
WBC	76	12.85	0.2	40.7	518	11.45	0.2	49	0.6008
Anion Gap	83	10.9	5	31	612	11.5	3.4	35	0.2973
Bicarb	83	20.3	8.6	29.3	609	21.1	10.9	37.4	0.0147
Gluc	83	106	29.3	216	608	108	17	249	0.8611
Length of Stay (days)	84	2	0	39	616	1	0	23	0.0095
Chi-square Test									
	n	%			n	%			
Admission							0.0221		
Home	24	28.57			257	41.72			
Floor	56	66.67			312	50.65			
PICU	4	4.76			47	7.63			

Discussion

Hyponatremic patients were found to have higher incidences of increased serum CRP as well as longer LOS. Lower bicarbonate levels were also correlated with hyponatremia as was

elevated white blood cell count. This was expected as the reabsorption of sodium in the kidneys is mechanistically related with the use of hydrogen protons. In the case of any infection, white blood cell proliferation will also be higher than normal. The elevation CRP confirms the inflammatory nature of sepsis and confirms its use as a predictive biomarker of sepsis.

Hyponatremic patients were found to be 1.79 times ($P = 0.0211$, 95% CI 1.08 – 2.95) more likely to be admitted for hospitalization when compared to those that were not hyponatremic.

Limitations

Some limitations to take into account included the selectivity of patients. The EPIC program was used to gather patients that were present in the ED with a fever and a final diagnoses of sepsis. If a child was present in the ED but did not have a BMP, CBC, or a CRP lab, they were also excluded from the study. However, patients included in the study were not required to have all laboratory assays. The span of dates as well as a limited location may have also influenced the outcomes. Because the percentage of hyponatremic patients was low, a reevaluation of the definition of sepsis may be enacted to encompass more patients, as literature values are variable in their definition. Lastly, the data is limited to application on children. Adults have different metabolic rates and respond to pathogens differently.

Conclusion

Sepsis continues to take the lives of children. The use of several biomarkers to predict pathogenic disease is becoming more common. As the literature expands on the mechanisms of action, the ability to predict and prevent the progression of septic shock will help save many lives. In our study, fever, hyponatremia and CRP were used to find a correlation between admission, discharge and length of stay. However, there was inconclusive evidence on the effectiveness of using these biomarkers as early predictive signs of a sepsis infection. Fever and CRP were

determined to be the best biomarkers to use in predicting a sepsis infection, as it was elevated in all patients. Hyponatremia proved to be present only in a small percentage (12%) of the population. However, literature values for hyponatremia are variable, and perhaps encompassing more patients a better correlation may have been established. By using a retrospective approach, a larger population of sepsis patients may be evaluated to pave the way to future hypotheses regarding sepsis and the way physicians diagnose it. Retrospective studies are valuable, as data can be quickly and efficiently collected, with no harm befalling the patient to draw a replicable conclusion. For example, hyponatremia patients were found to be almost twice as likely to be admitted to the hospital in a small sample size of 84. The use of inexpensive and replicable biomarkers that are routine in many emergency departments could help prevent life-threatening situations not only for sepsis, but for many infectious processes. The ability to diagnose the patient correctly ultimately falls on the judgement of the physician.

Acknowledgements

I am grateful to Dr. Osama El-Assal and Deborah Maglionico for their helpful assistance in teaching me how to conduct retrospective research. I thank Akron Children's Hospital and the Considine Research Center for allowing me to conduct my research project under their supervision. I also wish to acknowledge and thank my research project readers Dr. Brian Bagatto and Dr. Jennifer Cooper for facilitating my knowledge at the University of Akron. Lastly I would like to thank and acknowledge Dr. Jim Holda for putting me in contact with Deborah and the Considine Research center and reviewing my proposal.

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