Low Levels of Unsaturated Transferrin as a Predictor of Survival in Pneumococcal Pneumonia*

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ABSTRACT

A study was conducted to evaluate factors which might influence the prognosis of persons with pneumonia owing to Streptococcus pneumoniae. Serum iron, total iron binding capacity and unbound iron binding capacity (UIBC), were evaluated in 35 such patients. Of the 10 patients with UIBC levels less than 130 μg per dl on admission to the hospital, six died, three had complications, and only one recovered uneventfully. Of the 25 surviving patients, 21 (84 percent) had UIBC greater than 130 μg per dl on admission. Positive blood cultures were also correlated with survival. However, the strongest prognostic indicator was the combination of UIBC and blood culture results. Only 14 percent of patients with abnormalities in both parameters survived, whereas 80 percent to 88 percent of those with negative blood culture and/or normal UIBC survived. This suggests that insufficient unsaturated transferrin may facilitate bacteremia and contribute to lethality of pneumococcal pneumonia.

Introduction

The mortality of patients with pneumococcal pneumonia remains 10 percent to 20 percent despite numerous improvements in antibiotics, diagnostic procedures and supportive care. Although Streptococcus pneumoniae is almost always exquisitely sensitive to penicillin, patients who develop bacteremia have a poor prognosis, even without underlying disease and despite adequate chemotherapy. Various studies have identified bacteremia, leukopenia, chronic disease and age over 70 years as risk factors associated with mortality. Alcoholism, as a contributing factor, is controversial. It does predispose an individual to the disease, but it is not associated with increased mortality. The mechanisms by which these factors contribute to increased mortality has not been adequately explained.

Unsaturated transferrin has been implicated as the critical component of
serum that inhibits the growth of many bacteria and fungi. Transferrin is the major iron transport protein in plasma. It binds iron and carries it between stores in the liver and the reticuloendothelial system to tissues where it is needed. Nearly all bacteria require a continuous supply of exogenous iron to sustain growth. In normal serum, transferrin binds iron with such high avidity that it is unavailable for utilization by most microorganisms, rendering the serum bacteriostatic. Adequate binding of iron by transferrin has been demonstrated to be the decisive factor in determining survival in several experimental infections. Clinical studies in numerous groups of patients including those with leukemia and bone marrow transplantation have also implicated transferrin as an important host defense mechanism.

This investigation was designed to determine whether levels of iron and transferrin might predict clinical outcome in patients with pneumococcal pneumonia. Serum transferrin was measured by the indirect method of total iron binding capacity (TIBC) because it facilitated calculation of serum levels of unsaturated transferrin as unbound iron binding capacity (UIBC) which we predicted would be an important factor in determining prognosis.

Materials and Methods

Patient Selection

Serum samples were obtained from patients admitted to Grady Memorial Hospital, a large municipal hospital in Atlanta, Georgia, for community acquired pneumococcal pneumonia during a three month period January to March. The criteria used for patient selection and diagnosis of pneumonia attributed to Streptococcus pneumoniae were: (1) Roentgenographic evidence of pneumonia; (2) Isolation of Streptococcus pneumoniae from sputum culture as the sole pathogen; and (3) Clinical evidence of pneumonia. Patients who failed to meet all three criteria were excluded from this study.

At the end of the study, patient outcome was divided into three categories: (1) Normal recovery, defined as resolution of fever by the fourth hospital day and total hospitalization no longer than seven days; (2) Complicated recovery, defined as persistent fever beyond the fourth day after admission or hospitalization exceeding seven days; and (3) Death attributed to pneumococcal disease.

Sample Collection and Processing

Serial venous blood samples were obtained for patient care purposes from each subject admitted to the study. Excess serum which was not required for patient care was frozen at −85°C until analysis for this study. Values for TIBC and serum iron were determined by a ferrazine assay adapted to a centrifugal analyzer.* The UIBC was calculated from TIBC and percent of saturation. Percent of saturation was calculated as follows:

\[ \% \text{ saturation} = \frac{\text{serum iron}}{\text{TIBC}} \times 100 \]

UIBC was then determined by the following formula:

\[ \text{UIBC} = \frac{100 - \% \text{ saturation}}{100} \times \text{TIBC} \]

The level of UIBC of 130 μg per dl was selected as the value which best differentiated survivors from the patients who died. Blood samples from all patients were cultured for microorganisms as part of regular patient care.

* Cobas Bio, Roche.
The Fisher exact test was used to determine the significance of differences in survival among groups of patients. The Student’s T test was used to evaluate differences among means of test values.

Sensitivity, specificity and predictive value of a positive test were calculated by standard methods. The sensitivity is the probability of a correct result for persons known to have the disease. The specificity is the probability of a correct result for persons known to not have the disease. The predictive value of a positive test is the probability that a positive result will be associated with disease. Results

Thirty-five patients fulfilling the diagnostic criteria of the study were evaluated. The demographic data of the patient population grouped according to survival are displayed in table I. As has been noted in other studies of pneumococcal pneumonia, there was a difference in the mean age between the subjects who survived or succumbed to infection, but the range shows the older ages are represented in both groups. There were no significant differences between the two groups in mean hemoglobin, leukocyte levels, sex, or length of hospitalization. There was an increased incidence of culture positive bacteremia among the patients who died. The oxacillin screen demonstrated all isolates to be sensitive to penicillin. Consequently, antibiotic resistance probably did not contribute to mortality in this study.

Serum iron levels were low in all patients on admission. The difference in the mean serum iron levels between the two groups was not statistically significant. The admission transferrin levels were significantly lower in patients who died compared with those who recovered. The percent saturation was also low in both groups, but the means were not significantly different. The mean admission serum level of unsaturated transferrin was lower in those who died than those who recovered but the difference did not reach statistical significance (p < 0.1). Each of the values in table I was analyzed to determine its ability to predict prognosis. Blood culture and UIBC were found to be the most effective predictors of survival in this study (table II). The TIBC was less effective in predicting survival than UIBC even though differences in the mean values were of greater statistical significance. Positive blood culture and UIBC values of less than 130 μg per dl were of similar value in this group of patients as measured by sensitivity,
TABLE II

<table>
<thead>
<tr>
<th>Test results</th>
<th>Recovered</th>
<th>Died</th>
<th>P Value</th>
<th>Percent Recovery</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>7</td>
<td>&lt; 0.05</td>
<td>46</td>
<td>0.70</td>
<td>0.76</td>
<td>0.54</td>
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<tr>
<td>Negative</td>
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<td>3</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>UIBC‡</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt; 130)</td>
<td>5</td>
<td>7</td>
<td>&lt; 0.05</td>
<td>42</td>
<td>0.70</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td>Normal (≤ 130)</td>
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<td>3</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Blood culture and UIBC‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Low</td>
<td>1</td>
<td>6</td>
<td>&lt; 0.01†</td>
<td>14</td>
<td>0.60</td>
<td>0.96</td>
<td>0.86</td>
</tr>
<tr>
<td>Positive Normal</td>
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<td>2</td>
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</tr>
<tr>
<td>Negative Low</td>
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<td>1</td>
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</tbody>
</table>

*Statistics by Fisher exact test.  
‡Significance calculated for low unsaturated iron-binding capacity and positive blood culture (top line) versus either or both tests normal (three lower lines).  
§Unsaturated iron-binding capacity.

specificity and predictive value calculations. An abnormal value of either parameter predicted that over half (54 percent and 58 percent) of the patients would die. The combination of the two tests greatly improved the predictive value of a positive result to 0.86 while increasing the specificity to 0.96 and decreasing the sensitivity to 0.60. Only one of seven (14 percent) of the subjects with abnormalities in both tests at the time of admission survived. The results of UIBC and blood culture assays correlated positively with one another, but not as strongly as the combination correlated with survival. Seven of 12 (58 percent) of subjects with low UIBC values while only six of 23 (26 percent) of those with normal UIBC values had positive blood cultures.

The patients who recovered were subdivided into those who recovered uneventfully and those who suffered complications of prolonged fever or extended hospitalization. The UIBC values differentiated these two groups surprisingly well (figure 1). Eighteen of 19 (95 percent) of those who recovered without complications had UIBC levels greater than 130 µg per dl on admission. Nine of 10 (90 percent) of those with UIBC levels less than 130 µg per dl suffered complications or died while 18 of 24 (75 percent) of those with normal levels recovered uneventfully. These differences are all highly statistically significant.

![Figure 1](image-url)  
**Figure 1.** Admission UIBC values grouped according to outcome of disease. Each point represents the value of UIBC of a single patient. The shaded area indicates values below 130 µg per dl.
The analysis of samples obtained after admission was limited by the inability to collect samples at consistent intervals. The lowest UIBC value obtained from any of the post admission samples are shown in figure 2. The values of several patients who experienced uneventful recoveries increased after admission. The serum UIBC values of most patients who died or suffered complicated recoveries fell in days after admission. Five of the patients who died had UIBC levels below 130 μg per dl during the entire hospitalization. A sixth patient had an admission level of 201 μg per dl that rapidly decreased to 119 μg per dl prior to death. Of the remaining three patients in this group with admission UIBC greater than 130 μg per dl, one had a steadily declining UIBC, one had borderline levels with a decreasing slope, and one had levels from 163 μg per dl to 159 μg per dl with a slight negative slope.

Discussion

This paper reports correlations between low levels of unsaturated transferrin, UIBC, positive blood cultures and prognosis in patients with community acquired pneumococcal pneumonia. The correlation of positive blood cultures and poor prognosis has been noted in many previous studies of pneumonia. The relationships of low UIBC to prognosis and survival of patients with pneumonia have not been previously reported. However, there is abundant evidence that unsaturated transferrin is an important host defense mechanism.

The percentage of people in our study who died (28%) was larger than reported in other studies (10 to 20 percent). This could be due to statistical variation in a small study or differences in the study population. Our case acquisition procedures and stringent diagnostic criteria may have biased our study population in favor of more seriously ill patients. Potential subjects were identified by monitoring the sputum culture results of the diagnostic microbiology laboratory. When S. pneumoniae was identified as the sole pathogen in a sample, the patient’s record was reviewed to obtain additional information and serum in excess of that needed for tests that had been ordered was obtained from the clinical chemistry laboratory. These procedures would select patients with extensive laboratory work who may have been sicker. It is also possible that other studies underestimated the incidence of death owing to pneumonia.

The sensitivity and specificity of diagnostic tests do not vary with incidence of disease in a study population. Consequently the values reported in this study should be valid within statistical limits for other populations of patients. The predictive value of a positive test, however, would be lower in a population with lower incidence of death. Nevertheless, the incidence of death owing to S. pneumoniae pneumonia is sufficiently high in most studies to insure that a test with the sensitivity and specificity values...
Transferrin and Survival in Pneumonia

of the present study would have significant clinical value and to suggest that the underlying parameters have biologic significance.

Alterations in serum iron and transferrin occur as part of the acute phase response to infection. Serum iron levels characteristically drop precipitously with the initiation of infection or other inflammatory condition. This increases the level of unsaturated transferrin, UIBC, and increases the bacteriostatic capacity of serum. In several experimental models, injection of iron in quantities sufficient to saturate serum transferrin is able to transform self-limited infections into lethal ones even though other host defense mechanisms such as neutrophils, complement and immune responses remain intact. The reduction in serum iron associated with acute inflammation is initiated by leukocyte endogenous mediator (LEM), now known as interleukin-1 (IL-1). The IL-1 is secreted by mononuclear phagocytes in response to many microbial products and has numerous physiologic effects. A defect in the secretion of IL-1 or an over supply of iron could contribute to low levels of unsaturated transferrin.

Serum transferrin is reduced by malnutrition and chronic inflammation. Both serum iron and transferrin are depressed in chronic diseases including chronic infection, rheumatoid arthritis, neoplasia, certain hematologic disorders, and various hepatic and renal diseases. There is evidence that low levels of transferrin may potentiate bacteremia. Consequently, preexistence of low transferrin might be expected to promote bacterial proliferation in patients with acute pneumococcal pneumonia.

An unexplained feature of pneumococcal pneumonia is that a percentage of people die in spite of seemingly adequate antibiotic therapy and modern supportive care. The present study suggests that deaths may be related to low UIBC and an associated deficient bacteriostatic capacity of serum. Although much remains to be learned, the following hypothesis is consistent with available information. Without adequate bacteriostatic capacity, organisms might proliferate massively in the lung and blood stream prior to initiation of antibiotic therapy. Pneumococcal polysaccharide is able to activate complement and probably also other inflammatory mediators. It is resistant to digestion and persists in the body for long periods. If a large amount of polysaccharide or other toxin were produced prior to initiation of antibiotic therapy, it could intensify and prolong disease long after live organisms are eliminated. If it could be demonstrated that such factors contribute to morbidity and mortality of pneumococcal pneumonia, then more effective supportive therapy might be developed.

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References


