The Value of IL10, RANTES and Antithrombin in Prediction of Sepsis Induced DIC in Preterm Infants

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Abstract

Late onset bacterial infection and DIC and/or septic shock in preterm very low birth weight infants carry a high risk of morbidity and mortality. The progression to DIC in infected very low birth weight infants is difficult to predict at the onset of sepsis. We investigated the levels of IL10, RANTES and Antithrombin and the usefulness of their measurement to predict the development of sepsis induced DIC at onset of clinical presentation of infection. We investigated 60 infants with clinical signs and symptoms of sepsis, of which 22 were proven blood culture positive (group 1), 18 were clinically infected culture negative (group 2) and 20 non infected served as control group (group 3).

IL10 showed a significant up regulation in group 1 compared to group 2&3 and in the subgroup who developed DIC while RANTES and Antithrombin showed significant down regulation.

Our model which consisted of simultaneous measurement of IL10, RANTES and Antithrombin showed that IL10 at level >1400pg/ml, RANTES at level <2850pg/ml and Antithrombin at concentration <13.2mg/dl could sensitively and reliably predicted the development of all DIC patients in septic infants at onset of clinical presentation without misdiagnoses of non DIC cases. This information could be vital for early and effective treatment of neonatal sepsis.

Key Words: DIC – Septic shock – Preterm – RANTES – Antithrombin.

Introduction

PRETERM birth refers to the birth of a baby of less than 37 weeks gestational age. Late onset (>72 hours of age) infection in preterm continues to be an important cause of morbidity and mortality in very low birth weight (<1 500 gram) infants [1]. The immunological defense mechanisms in preterm may be immature and/or deficient which predispose them to serious infections [2].

Early warning signs of late onset bacterial infections are often non specific, subtle and inconspicuous, but the clinical course may be alarmingly fulminant leading to septic shock, disseminated intravascular coagulation and death within hours of onset [3].

Much basic and clinical research has been focused on the inflammatory cascade in sepsis and the use of inflammatory mediators for early diagnosis and outcome prediction [4].

It is known that acute inflammatory reaction can exert dual influences on patients with sepsis, chemokines and proinflammatory cytokines are essential for host defense against microbial infection, but excessive influx of activated leucocytes coupled with exaggerated production of potent proinflammatory mediators can lead to deleterious consequences leading to widespread small vessels damage multiorgan dysfunction and death [5].

RANTES (Regulated upon activation, Normal Tcell Expressed and presumably Secreted) as known as CCL5 is a member of CC subfamily of chemokines and plays a primary role in the inflammatory immune response via its ability to chemoattract leucocytes and modulate their function [6].

IL10 have counterregulatory properties that can down regulate the release and effect of proinflammatory mediators [7].

Antithrombin (an endogenous anticoagulant) is a potent inhibitor of thrombin-mediated vascular injury in the micro-circulation during severe sepsis [8].

DIC is a syndrome characterized by a systemic activation of coagulation leading to intravascular deposition of fibrin in the (micro) vasculature and
The Value of IL10, RANTES & Antithrombin in Prediction

DIC in severe infections is thought to be triggered by endotoxins and other microbial mediators from pathogenic microorganisms which activate coagulation pathways via proinflammatory cytokines. Presence of DIC plays a role in organ failure and is an important predictor of mortality [9].

Aim of the work:
This study aimed to: (1) Measure levels of IL10, RANTES and Antithrombin in preterm very low birth weight infants with sepsis and to quantitatively compare the magnitude of their response in severely infected infants who subsequently developed DIC with the response in less seriously infected infants without DIC. (2) Investigate the usefulness of measurement of IL10, RANTES and Antithrombin to predict the development of sepsis induced DIC at onset of clinical presentation of infection.

Early identification of infants with severe infection and DIC could enable neonatologists to pay special attention to patients who are most at risk for serious complications.

Subjects and Methods
This study had been conducted on sixty preterm very low birth weight infants in the neonatal intensive care unit at El Galaa Teaching hospital after receipt of patient consent.

Inclusion criteria included:
Birth weight <1500gm and postnatal age >72 hours and with signs and symptoms suggestive of systemic infection (fluctuation of temperature, lethargy, irritability, respiratory dysfunction, gastrointestinal dysfunction or cardiovascular dysfunction).

Patients who were already receiving antibiotics at time of sepsis evaluation, or had severe congenital abnormalities were excluded from the study.

All patients were subjected to:
• Full history taking and thorough clinical examination.
• Chest and abdominal radiography.
• 10ml of blood sample was taken at the time of clinical presentation of suspected sepsis before any start of antibiotics.

1ml blood was placed in a pediatric blood culture bottle for blood culture.

2ml was put on EDTA tube for complete blood counts by sysmex.

1.8ml on citrated test tube (9 volume blood: 1 volume citrate) for antithrombin.

The left blood was left to clot, centrifuged and serum obtained were stored at −70 until assay of IL10 and RANTES.

Measurement of RANTES and IL10 occurred by ELISA technique using Quantikine immunoassay kits.

Measurement of antithrombin occurred by radial immunodiffusion kit.

Follow-up the patients occurred by:
• Complete blood counts and differential count.
• Serial CRP measurement by CRP latex (cromatest).
• Coagulation study to detect development of DIC.

By: *Serum D-dimer concentration by latex agglutination.

*Prothrombin time and activated partial thromboplastin time using clot 2S.

According to the results of blood culture, patients were further subdivided into three groups:

Group 1: The infected group, included infants with sepsis episode that had been confirmed as microbial culture positive sepsis including septicemia.

Group 2: Clinically infected culture negative group included infants with sepsis episode with at least 3 clinical signs and symptoms suggestive of clinical sepsis or chest radiographic features suggestive of pneumonia and serial CRP concentration >6mg/l. This group was classified as true infections based on their strong and persistent clinical signs of sepsis.

Group 3: The non infected group included infants who met the initial criteria for suspected clinical sepsis but were classified as non infected and antibiotic stopped early after improvement (served as control group).

Group 1 (the infected group) were further subclassified according to the development of DIC into:

DIC subgroup: With increased serum D-dimer concentration >1.0mg/l, thrombocytopenia <100x10⁹ cells/l and prolonged activated partial thromboplastin time >120s.
Non DIC subgroup: With normal [serum Ddimer (0.5-1.0mg/l), platelet count and activated partial thromboplastin time (26-40s)].

**Statistical analysis:**
Data analysis was performed using SPSS windows version 10. *p* values <0.05 was considered significant.

**Results**
A total of 60 suspected infection episode were investigated of which 22 were classified into the infected proven blood culture positive (group 1) of which 9 were gram positive, 5 gram negative and 8 mixed infection septicemia 18 clinically infected culture negative (group 2) and 20 into the non-infected (group 3) which served as control group.

There were no significant statistical differences among the groups as regards gestational age or birth weight.

In the infected group (group 1), 9 infants developed DIC and none of the cases in groups 2 and 3 developed DIC.

In the DIC group, 5 cases were attributable to gram positive septicemia, 2 cases to gram negative septicemia and 2 cases to mixed infection septicemia, while in the non DIC group, infants with infection attributable to mixed infection septicemia were 6 cases, to gram positive septicemia 4 cases and to gram negative 3 cases.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>No. of cases (n=60)</th>
<th>DIC cases</th>
<th>Non DIC cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) Clinically infected Positive culture septicemia</td>
<td>22 (37%)</td>
<td>9 (41%)</td>
<td>13 (59%)</td>
</tr>
<tr>
<td>Group (1) Gram positive</td>
<td>9 (15%)</td>
<td>5 (56%)</td>
<td>4 (44%)</td>
</tr>
<tr>
<td>Group (1) Gram negative</td>
<td>5 (8%)</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Group (1) Mixed infection</td>
<td>8 (14%)</td>
<td>2 (25%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Group (2) Clinically infected Negative blood culture</td>
<td>18 (30%)</td>
<td>0 (0%)</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>Group (3) Non infected (control group)</td>
<td>20 (33%)</td>
<td>0 (0%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

DIC = Disseminated intravascular coagulation.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Gestational age (weeks)</th>
<th>Birth weight (kg)</th>
<th>RANTES (pg/ml)</th>
<th>IL10 (pg/ml)</th>
<th>Antithrombin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>30.7±0.81</td>
<td>1.18±0.14</td>
<td>3884.5±1783.9</td>
<td>2175±1109.2</td>
<td>12.56±3.22</td>
</tr>
<tr>
<td>Group (2)</td>
<td>30.84±0.82</td>
<td>1.19±0.16</td>
<td>9200.9±1801.3</td>
<td>610.9±181.1</td>
<td>16.38±4.46</td>
</tr>
<tr>
<td>Group (3)</td>
<td>30.65±0.75</td>
<td>1.18±0.13</td>
<td>13977±1518.4</td>
<td>255.9±56.7</td>
<td>22.38±0.59</td>
</tr>
<tr>
<td><em>p</em> Group (1) versus Group (2)</td>
<td>0.70</td>
<td>0.90</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>p</em> Group (1) versus Group (3)</td>
<td>0.75</td>
<td>0.96</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>p</em> Group (2) versus Group (3)</td>
<td>0.80</td>
<td>0.89</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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<tr>
<th>Criteria</th>
<th>Gestational age (weeks)</th>
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<th>IL10 (pg/ml)</th>
<th>Antithrombin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIC group (n=9)</td>
<td>30.78±0.88</td>
<td>1.21±0.14</td>
<td>2288.9±396.7</td>
<td>3172±223.7</td>
<td>10.54±1.7</td>
</tr>
<tr>
<td>Non DIC group (n=13)</td>
<td>30.67±0.79</td>
<td>1.16±0.16</td>
<td>4989±1494.2</td>
<td>1484.6±926</td>
<td>13.95±3.3</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.758</td>
<td>0.969</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
### Table (4): Values for predicting the development of DIC in severely infected infants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Threshold level</th>
<th>Sensitivity (95% C.I.)</th>
<th>Specificity (95% C.I.)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL10</td>
<td>&gt;1400 (pg/ml)</td>
<td>100.0</td>
<td>92.7</td>
<td>0.972</td>
</tr>
<tr>
<td>RANTES</td>
<td>&lt;2850 (pg/ml)</td>
<td>100.0</td>
<td>95.1</td>
<td>0.980</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>&lt;13.2 (mg/dl)</td>
<td>100.0</td>
<td>83.0</td>
<td>0.925</td>
</tr>
</tbody>
</table>

AUC = Area under the curve.

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**Discussion**

The prediction and diagnosis of sepsis in preterm infants, especially very low birth weight are among the most serious problems in modern neonatology. Late onset bacterial infection and septic shock carry a high risk of morbidity and mortality as early warning signs and symptoms are often nonspecific and subtle and may be similar to various non infection conditions [4].

In recent years, cytokines and chemokines have been purposed as markers for diagnosis of infection in adults, children and infants and the interaction between these inflammatory mediators in response to sepsis remains a controversial subject [10].

*In our study, According to the results of blood culture, patients were divided into three groups:*

- **Group 1:** The infected group—culture positive septicemia
- **Group 2:** Clinically infected culture negative
- **Group 3:** The non infected—serving as control group

When comparing the three groups as regards gestational age and birth weight, no statistical significant differences were observed which were similar to results obtained by Ng et al. [11]. When investigating the immunological profile of preterm with sepsis and to Ersoy et al. [8] when investigating initial antithrombin levels in diagnosis and prediction of neonatal sepsis.

Serum IL10 was statistically significantly increased in group (1) when compared to group (2) or group (3) where $p=<0.001$ and in group (2)
When compared to group (3) p=<0.001. These results were in accordance to Ng et al. [11] when measuring a panel of chemokines and cytokines at 0 and 24 hours after clinical presentation in very low birth weight infants with suspected infection and concluded marked upsurge of anti inflammatory IL1 0 response suggesting that the counter regulatory mechanism was likely to be functional in preterm infants of early gestation so that those preterm were capable of eliciting a prominent inflammatory and anti inflammatory responses to invading pathogens. IL1 0 has the ability to suppress the synthesis of proinflammatory cytokines from Tcells, leucocytes and macrophages and effectively down regulates the proinflammatory responses [12].

When serum RANTES concentrations were compared between the three groups, there was a statistically significant decrease in group (1) when compared to either group (2) or group (3) p<0.001 and in group (2) when compared to group (3) p<0.001. Similar to our results, Ng et al. [11] have found that circulating RANTES was decreased significantly in preterm VLBW during the septicemic process and necrotizing enterocolitis. This observation is in accordance with findings that circulating RANTES were inversely correlated with the APACHE II score [8], plasma lipopolysaccharide concentrations [13] and adverse outcomes [5].

Antithrombin levels were significantly decreased in group (1) when compared to either group 2 or group 3 and in group 2 when compared to group 3. Our findings were supported by Ersoy et al. [8] who demonstrated that lower antithrombin levels in neonatal sepsis at time of clinical presentation were associated with disease severity and was a useful predictor of clinical outcome in neonatal sepsis. Similarly Lauterbach et al. [14] when examining the prognostic value of antithrombin and protein C in 150 neonates with late onset sepsis concluded that both antithrombin and protein C were significantly lower in neonates with sepsis either confirmed or not confirmed by blood culture and that lowest values were observed in neonates who had died with adverse outcomes in the course of sepsis.

When group (1) infected culture proven septi cemia were further subdivided according to development of DIC into: DIC subgroup and non DIC, serum IL1 0 were statistically significantly increased in infected infants with DIC when compared with the corresponding concentration in non DIC subgroup, in contrast serum RANTES and Antithrombin concentration were significantly lowered in the former subgroup.

In a study done by Ng et al. [11] they found intense induction of IL6 and IL10 by infection signals such as endotoxins and bacteria or their products in the sickest infants, where median plasma IL6 and IL1 0 concentrations in patients with DIC at 0h were at least 60 and 12 fold higher, respectively, than the corresponding concentration in infants without DIC. These characteristics rendered the two inflammatory mediators particularly useful in differentiating severely ill infants from less seriously infected patients at an early stage [11,15].

Ellis et al. [16] have attributed the significant decrease in RANTES concentration in DIC subgroup to the thrombocytopenic state associated with DIC, as platelets have been found to be a rich source of RANTES. Platelets surfaces express CD40 costimulatory molecules, which interact with CD154 counter-receptor on activated T lymphocytes thereby triggering the release of preformed RANTES [4].

When comparing Antithrombin levels in both DIC and non DIC subgroup, it showed a statistically significant decrease in the former subgroup. Erosy et al. [8] have reported that newborns with sepsis with lower antithrombin and fibrinogen levels had a higher rate of developing DIC and/or septic shock. They concluded that when sepsis is suspected in neonates with low levels of antithrombin, antithrombin replacement therapy may improve the prognosis because low levels are associated with increased risk of mortality.

A comparison of each individual test using the optimal cut off values showed that serum RANTES at concentration <2850pg/ml achieved a sensitivity of 100% and specificity of 95.1% to predict the development of DIC in septic infants at the onset of clinical presentation, IL10 at cut off level > 1400pg/ml had a sensitivity 100% and specificity 92.7% while Antithrombin <13.2mg/dl had sensitivity of 100% and specificity of 83.0%. Our study demonstrated that simultaneous measurement of the three parameters at 0h of sepsis evaluation could identify all the DIC patients without misclassification of any non DIC cases.

This is the first model to use the three parameters together of IL1 0, RANTES and Antithrombin to sensitively and reliably predict DIC at onset of clinical sepsis. Comparing the analysis of this result with a model using CRP results [3], the most
commonly used infection marker, CRP at concentration >15.5mg/l at 0h had sensitivity of 79% and specificity 78% for identifying DIC cases.

Ng et al. [11] had used CART model of IL10, IL6 and RANTES to predict the development of DIC at the very first signs of sepsis and found that IL10 >208ng/l and IL6 > 168ng/l and RANTES <3110 could identify all DIC cases but misclassified 5 non DIC cases.

In conclusion, our findings suggested that IL10 were markedly up regulated in very low birth weight infants with septicemia and in those who progressed to DIC while RANTES and Antithrombin were significantly down regulated.

Thus quantitative measurements of these inflammatory mediators at the onset of clinical presentation could assist neonatologists in predicting the severity of infection and DIC, thereby identifying seriously ill infants who are most in need of urgent treatment and targeting those who are most at risk of adverse sequelles. Moreover, supportive treatment that targets DIC may be essential in reducing organ dysfunction and mortality.

Finally, the mechanism and role of cytokines and chemokines in neonatal sepsis warrants further investigation and it would be particularly valuable to broaden the panel of studies on large scale and longer periods of follow up and we hope that these mediators could be used routinely so as to improve clinical management and treatment outcomes.

References