Evaluation Of Serum C-reactive Protein In Diagnosis And Prognosis Of Neonatal Septicemia

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Author(s): Kumar B

Abstract

Background: Neonatal septicemia is an overwhelming systemic infection due to gain access of pathogenic bacteria in the blood stream of neonate. C-reactive protein production is very early and sensitive response to microbial infections and has highest sensitivity, specificity and positive predictive accuracy in cases of neonatal septicemia.

Methods: Total 50 clinically suspected cases and 25 healthy neonates as control were studied. Total WBC count, absolute neutrophils count, Band Cell/Neutrophil Count ratio, C-reactive protein estimation, blood culture, culture of umbilical discharge, other purulent material and urine were done. CRP levels were noted on the day of admission before instituting therapy and 5th and 10th day of treatment.

Results: 22 cases were bacteriologically positive. CRP was positive in 44 cases and one control. Thirty-five cases were survived and 15 expired. CRP value among survivors on 1st, 5th and 10th day was 18.51+/-4.21, 12.61+/-3.51 and 8.21+/-3.05 respectively; whereas, on 1st and 5th day among expired was 23.22+/-2.42 and 21.42+/-3.21 respectively. Mean CRP value on the 1st day and 10th day in cured survivors was 17.89+/-4.52 and 6.0+/-7.0 (p value Conclusion: Serum CRP estimation is very sensitive and reliable method. Serial measurements of serum CRP levels are useful in monitoring the course and it provides an early indication of response of treatment. It can help in decision of initiating or discontinuing antibiotic therapy. The persistence or insignificant decline of serum CRP with treatment signifies about inadequate treatment or development of complications.

Key words: neonatal septicemia, C-reactive protein, blood culture, total leucocyte count, absolute neutrophil count, band cell/ neutrophil count ratio.

Introduction

Neonatal septicemia is an overwhelming systemic infection due to gain access of pathogenic bacteria in the blood stream of an infant upto the age of 28 days of life1. The gold standard for diagnosis of neonatal septicemia is isolation of microorganism from blood and site of infection. However, it is time-consuming procedure, requires well-equipped laboratory, trained personnel and success rate is merely 40-50%. Hence, the diagnosis is missed or delayed. These factors stress the need for some early diagnostic measures with reasonable specificity and sensitivity. Therefore, for early initiation of therapy, certain positive indirect markers along with clinical diagnosis are essential. For this purpose, total leukocyte count(TLC), absolute neutrophils count(ANC), Band cell to total neutrophils count (BC/NC) ratio, Micro-ESR and Acute phase reactants like C-reactive protein(CRP), Alpha1-acid glycoprotein and Alpha1-antitrypsin etc. have come in procedure2. Among these tests, CRP has highest sensitivity, specificity and positive predictive accuracy in neonatal septicemia3.

Materials And Methods

The present study was done in the department of Pathology and department of Microbiology of our medical college hospital. Cases and controls were selected from the department of Pediatrics and Obstetrics respectively. Fifty clinically suspected cases of septicemia comprised for study group and 25 healthy neonates served as control group. All the cases were studied under following headings.

1. Laboratory Investigations:

A) Blood Examination:

a) TLC;

b) Differential leukocyte Count(DLC), ANC and estimation of BC/NC ratio;

c) Culture and sensitivity(C/S) of blood;

d) CRP estimation on the day of admission, on the 5th and 10th day of treatment.

B) C/S of umbilical discharge, pustules or abscess when present.

C) Urine C/S as per requirement.

2. Procedures:

a) TLC: Capillary blood from free flowing heel prick
was taken up. TLC was done with the help of Haemocytometer.

b) DLC, ANC and BC/NC ratio: Blood smears were made on clean glass slides, air dried and stained by Leishman stain. The cells were examined under oil immersion lens. The differential count was done in two sets of 100 white blood cells. From the percentage of cell type, the ANC per cmm was calculated. BC/NC ratio was obtained after identifying the band cell in peripheral smear. Band cells were identified by cells with no lobulation for those in which the nucleus was indented but isthmus between the lobes was wide more than one third of the broadest segment.

The findings were classified as abnormal using following criteria:

1. TLC <5,000/cmm.
2. ANC suggestive if counts were either < 1000/cmm or >10,000/cmm.
3. BC/NC ratio >0.2.

b) Blood Culture:
Sampling Technique: 2ml of Blood samples were obtained from the prominent vein by scalp vein set and disposable syringe after taking aseptic precaution. It was added to liquid glucose broth contained in a flask. The flask was plugged and rotated to mix the blood and broth thoroughly.

Culture: The flask was incubated at 37°C under aerobic conditions. After 24 hrs, subcultures were done. Depending upon the type of organism, it was inoculated in a) Nutrient agar b) Blood agar c) MacConkey medium d) Wilson Blair medium and again incubated for 24hrs at 37°C.

c) Culture of umbilical discharge and other purulent material: The samples for culture were taken with sterilized cotton swab stick from the affected site. After taking sample, stick was quickly placed inside a sterilized autoclaved culture tube. Culture was done on different media in usual manner.

d) Urine culture: The genitalia were cleaned with savlon solution and mid stream urine was taken in culture tube and inoculated as usual manner for C/S.

Identification of organism from culture: Types of bacterial growth from culture were identified on the basis of:

a) Morphology of colonies.
b) Type of staining reaction such as Gram positive or negative.
c) Shape and other morphology of organism whether in pairs, chains or other groups.
d) Motility of organism- Whether motile or nonmotile. Motile- E.coli, Proteus, Pseudomonas etc. Nonmotile- Klebsiella, Shigella etc.
e) Growth of organism in a particular media- e.g. Wilson and Blair media- Salmonella.
f) Type of hemolytic reaction in blood agar media e.g. Beta hemolysis by Staphylococcus, group B Streptococcus, Streptococcus fecalis etc.
g) Type of colony in MacConkey media- whether lactose fermenter or nonfermenter. Lactose fermenter: - produce bright pink colonies e.g. E.coli, Klebsiella etc. Lactose nonfermenter- produce colorless colonies e.g. Proteus, Pseudomonas, Salmonella, Shigella etc.
h) Different biochemical reactions such as-

1. Sugar fermentation.
2. Tests to differentiate among enterobacteriace e.g. indole production, Methyl red, Voges-Proskauer reaction and citrate utilization tests.
3. Some specific reactions for organisms e.g. phosphate reaction- Staphylococcus aureus; hydrolysis of hippuric acid- Group B Streptococcus; deamination of phenyl alanine to phenylpyruvic acid-Proteus; arginine hydrolyase reaction- Pseudonymous, Salmonella etc. pigment production- Pseudomonas; Staphylococcus aureus. Antibiotic sensitivity was determined by disc diffusion technique.

Estimation of serum CRP- CRP was estimated by slide latex agglutination method. The reagents and information regarding their use were provided in the kit HUMAN'S HUMATAX CRP (German Product).

Principle- The CRP reagent had latex particles coated with anti-human CRP antibody. When the reagent was mixed with serum containing CRP at a level greater than 6mg/L, the particles agglutinated. This was interpreted as being positive sample. The reagent was also used for the semi-quantification of CRP. For this purpose the sample was diluted over a range of dilutions and each was tested qualitatively. The CRP level was estimated from the last dilution with visible agglutination.

Sensitivity: Humatax was standardized to detect CRP concentrations in non-diluted serum samples of approximately 6mg/L or higher.

Contents, reagents and materials provided:

1. CRP latex reagent (blue) (white cap);
2. 1ml control serum positive (red cap);
3. 1ml control serum negative (green cap);
4. 1 slide with 6 cells. Reagent 1, 2 and 3 contained 0.095% sodium azide as a preservative.
**Stability:** Latex reagent and control sera were stable up to the expiry date when stored at 2-8°C.

**Serum sample:** 2 ml blood was obtained by venipuncture into a sterile vial without anticoagulant. It was allowed to clot at room temperature and after complete clot retraction, serum was separated for testing. The serum sample was stored in refrigerator at 2-8°C.

**PROCEDURE: SLIDE TEST:**

A] **Qualitative determination (screening test):** Latex reagent, control and serum samples were brought to room temperature. Mixed the latex reagent prior to use to suspend the latex particle completely. Pipetted reagent onto separate cells of the slide. Serum sample- 40µL, control serum positive bottle-1 drop, Control serum negative bottle- 1 drop, CRP latex reagent- 1 drop each into all samples and control cells were applied. Mixed with separate sticks and spreaded the fluid over the entire area of the particular cells. Tilted the slide back and forth for 2 minutes so that the mixture was rotated slowly. At the end of 2 minutes results were read.

**Interpretation of results:** Distinct agglutination indicates a CRP content of more than 6mg/L in the non-diluted specimen. Sera with positive results were retested in the titration test.

B] **Semiquantitative test:** diluted the specimen with glycine NaCl buffer. Positivity in 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8...... dilution equals to 12, 18, 24, 30, 36, 42 and 48..... mg/L of CRP value in undiluted sample.

**Quality control:** Positive and negative control sera were used with each series. Their results were compared with those of the unknown specimen to distinguish possible granularity from agglutination. The positive control showed a distinct agglutination within 2 minutes. The negative control showed a smooth suspension without any visible agglutination after 2 minutes.

**CRP level:** CRP was positive in 44 cases and 1 control (Table 2). CRP was positive in 44 cases and 1 control (Table 3). Thirty-five cases were survived and 15 expired. CRP value among survivors on 1st, 5th and 10th day was 18.51+/-.4.21, 12.61+/-3.51 and 8.21+/-.3.05 respectively; whereas, on 1st and 5th day among expired was 23.22+/-.2.42 and 21+/-.3.21 respectively (Table 4). Mean CRP value on the 1st day and 10th day in cured survivors was 17.89+/-4.52 and 6.0+/-7.0 (p value <0.001) whereas in complicated cases among survived was 24.00+/-7.0 and 16.21+/-.4.21 respectively (Table 5). Leucopenia was found in 22 cases (Table 6). Absolute Neutrophil Count was suggestive in 30 cases (Table 7). Band cell/ Neutrophil count ratio was found abnormal in 34 cases (Table 8). Sensitivity, Specificity and Positive predictive accuracy of CRP; TLC; ANC; BC/NC ratio were 90.90%, 96.00%, 95.23%; 45.45, 100.00, 100.00; 63.63, 64.00, 60.68; 72.72, 68.00, 66.67 respectively (Table 9 and Graph 1).

**Discussion**

Neonatal septicemia is very common in the present Indian set-up. The disease has got high morbidity and mortality but it is unfortunate that none of laboratory parameters available till are rapid, specific, sensitive, cheap and simple enough to confirm the diagnosis and to assess the prognosis or therapeutic response in this condition. The present work was concluded to assess the efficacy and reliability of CRP in neonatal septicemia and values of the CRP as a tool of prognosis in neonatal septicemia. CRP production is very early and sensitive response to most form of microbial infections. CRP is found in very low concentration in the sera of healthy neonate. Its concentration raises upto 3,000 fold in response to most forms of tissue damage, inflammatory conditions and infections. It returns to its normal level when inflammatory reaction subsides. Fetus as early as 28 weeks of gestation have high concentration of CRP in serum in response to variety of infections. It doesnot crosses placenta. Its concentration in mother and fetus are independent of each-other. A good response to antibiotics is indicated by rapid decline in level of CRP whereas persistent elevation of serum CRP suggests either the treatment is inadequate or some complication have developed. CRP has considerable utility for predicting outcome in terms of survival or complications. Commonly used anti-inflammatory or immunosuppressive drugs including steroids, unless
these drugs affect actively of underlying diseases do not affect the CRP response. After 3 days of life, CRP is the best single test in early detection of neonatal septicemia. Serial serum CRP estimation confirms the diagnosis; monitor the course of infection and the efficacy of antibiotic treatment. In episodes of neonatal septicemia, where, antibacterial treatment fails, CRP levels are moderately elevated, the day prior to treatment start and increased continuously thereafter. Whereas, success of treatment is generally accompanied by a decline in CRP within four days. Serum CRP evaluation by semiquantitative latex agglutination technique is quite rapid, giving result in 2-4 minutes, reliable, cheap and does not require a big sophisticated laboratory, rather it can be done in small laboratory.

A large number of methods are available for the determination of CRP in the serum. Although, electroimmunoprecipitation assay, immunometric assay and laser nephelometry are sensitive and quantitative method for the estimation of CRP. The facilities for such specialized investigations are not available at all centres. In their absence, latex agglutination method is quiet sensitive, quick and semiquantitative method for CRP determination. In the present study serum CRP was done by rapid slide latex agglutination method semiquantitatively and the concentration of 6 mg/L or more was considered as positive similar to other study. 25 healthy neonates as a control group were selected. The result of the test showed that the serum was positive for CRP in only 4% of the control cases, which was followed up for a period of 4 weeks and not showed any infection. Similar observation was made in other studies. 22 (44%) cases were found culture positive and 28(56%) negative. These findings correlated with the findings of other studies. However higher percentage of positive cases were also observed in the present study, Gram negative organisms were found in 77.27% cases. Escherichia coli was the commonest organism isolated, followed by Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Group B Streptococcus and Streptococcus fecalis similar to other study. However, Klebsiella pneumoniae or Staphylococcus aureus was isolated as the most common organism in early onset and 67% in late onset septicemia was also found. Mean value of CRP in the present study group was 20.31+/-4.27% on the first day. This finding reinforce the different study where mean value of serum CRP was 15.75+/-12µg/ml in blood culture positive and 6.13+/-11.72µg/ml in culture negative cases. The mean CRP values in positive cases, which survived and expired, were 18.51+/-4.21 and 23.22+/-2.42mg/L, 12.61+/-mg/L and 21+/-3.21mg/L on the 1st and 5th day respectively. On the 10th day it was found 8.21+/-3.05 in neonates who survived. After that, CRP was positive only in 17 out of 35 survived cases. It was observed that initial value of CRP were just significantly different (P<0.02) in both survived and expired groups similar to other study. With therapy CRP decreased in both survived and expired group on 5th and 10th day recording, but, fall was highly significant (P<0.001). Clinical improvement was noted in all cases where CRP had becoming negative. The above observations made, confirm the utility of CRP as a laboratory diagnostic and prognostic tool in following the course of neonatal septicemia. A good response of treatment was assessed by rapid fall in CRP level whereas insignificant rise of CRP suggested that either the treatment was inadequate or some complications had developed. Similar observations was made in different study, in which maximum level of CRP reached within one to two days of onset of infection and disappeared after one to two days of recovery. Serial determination of CRP was found useful in monitoring the course of pyogenic infections. CRP levels, which did not fall to normal within 7-10 days, remained elevated or increased again were usually a warning sign of a complications. Significant decline of CRP was found as early as 3rd day among survivors but not among those who expired.

Out of 35 cured cases, six developed complications. They were not responded well to treatment. First day mean CRP levels among the complicated patients were significantly higher than those who got clinically cure. In cured patients it was 17.89+/-4.52mg/L while in complicated patients it was 24.00+/-7.0mg/L.
Similarly in the third reading on 10th day decline was much more in cured patients than in complicated patients. Similar to our observations was found by other workers. Sensitivity, specificity and positive predictive accuracy of CRP were assessed. For comparison the sensitivity of total leucocyte count (TLC), absolute neutrophil count (ANC) and band cell/neutrophil count (BC/NC) ratio were also assessed. Sensitivity, specificity and positive predictive accuracy for CRP were 90.90%, 96.00 and 95.23% respectively; while for TLC, ANC and BC/NC ratio they were markedly less. Total leucocyte count (leucopenia) gave indication of infection in 44% of cases while no leucopenia was found in control group. Absolute neutrophil count was suggestive in 60% in study group and 36% in control group. BC/NC ratio> 0.2 was present in 68% and 32% in study group and control group respectively. TLC in 71.9% and BC/NC ratio in 69.4% of cases were found suggestive by others.

Immature to Total (I/T) ratio was found very sensitive indicator (71%). Sensitivity and specificity of leucopenia with I/T ratio was found 67%/90% and 78%/73% in first 3 day which was good parameter, and after 3 days 84%/66% and 79%/47%. Leucopenia and BC/NC ratio more useful than CRP were also suggested. Use of CRP for differentiation between positive and contaminated blood cultures in children and as better predictor than WBC or ANC were also proposed. However, both CRP and WBC were found useful for the diagnosis of late neonatal sepsis and accuracy increased when CRP and WBC were combined and sequential CRP assay results were used.

Conclusion

Serum CRP is very sensitive and reliable method for septicemia in neonates. Serial measurements of serum CRP levels are useful in monitoring the course of neonatal septicemia. It provides an early indication of response of treatment. It can help in decision of initiating or discontinuing antibiotic therapy. The persistence or insignificant decline of serum CRP with treatment signifies about inadequate treatment or development of complications.

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I acknowledge Dr (Prof) Basanti Verma, former head of the department of Pathology and Dr. R. K. Mahapatra, former head of the department of microbiology, Darbhanga Medical College, Laheriasarai for their guidance during the study.

References

Illustrations

Illustration 1

Table 1: Bacteriological profile in cases with its classification

<table>
<thead>
<tr>
<th>Features and classification of cases</th>
<th>number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriologically -ve – <strong>Probable case</strong></td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td>Bacteriologically +ve (44%)-- <strong>Confirmed cases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive in blood</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Positive in urine</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Positive in umbilical discharge</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Positive in other purulent discharge</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Illustration 2

Table 2: Bacteriological profile in culture positive case

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of cases (n=22)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative (77.37%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>31.81</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>6</td>
<td>27.37</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
<td>13.64</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>4.55</td>
</tr>
<tr>
<td><strong>Gram positive (22.73%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>13.63</td>
</tr>
<tr>
<td>Group B Sreptococcus</td>
<td>1</td>
<td>4.55</td>
</tr>
<tr>
<td>Strptococcus fecalis</td>
<td>1</td>
<td>4.55</td>
</tr>
</tbody>
</table>
Illustration 3

Table 3: Serum CRP profile in controls and cases

<table>
<thead>
<tr>
<th>CRP Profile</th>
<th>Control group</th>
<th>Study group</th>
<th></th>
<th></th>
<th>CRP level(mg/L), mean+/- S.D</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number  %</td>
<td>Number %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP+ve</td>
<td>1  4</td>
<td>44  88</td>
<td></td>
<td></td>
<td>20.31+/-4.27</td>
<td>28.8</td>
</tr>
<tr>
<td>CRP-ve</td>
<td>24  96</td>
<td>6  12</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Illustration 4

Table 4: Serial estimation of CRP in survivors and expired neonates

<table>
<thead>
<tr>
<th>Group</th>
<th>CRP Profile</th>
<th>CRP value mean +/- S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day (before treatment)</td>
</tr>
<tr>
<td>Survivors</td>
<td>CRP+ve (n=29)</td>
<td>18.51 +/- 4.21* (n=35)</td>
</tr>
<tr>
<td></td>
<td>CRP-ve (n=6)</td>
<td></td>
</tr>
<tr>
<td>Expired</td>
<td>CRP+ve (n=15)</td>
<td>23.22 +/- 2.42 (n=15)</td>
</tr>
<tr>
<td></td>
<td>CRP-ve (n=0)</td>
<td></td>
</tr>
</tbody>
</table>

P value:
*/** <.001  highly significant
*/*** <.001  highly significant
Illustration 5

Table 5: Comparison of 1st reading and 3rd reading in cases with complications among survivors.

<table>
<thead>
<tr>
<th></th>
<th>First reading on 1st day</th>
<th>Third reading on 10th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cured (n=29)</td>
<td>With complications (n=6)</td>
</tr>
<tr>
<td>CRP mean +/- S.D</td>
<td>17.89 +/- 4.52*</td>
<td>24.00 +/- 7.0**</td>
</tr>
<tr>
<td>(mg/L)</td>
<td>(n=11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.00 +/- 7.0</td>
<td>16.21 +/- 4.21</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td></td>
</tr>
</tbody>
</table>
Illustration 6

Table 6: Total leukocyte count profile of the study and control group

<table>
<thead>
<tr>
<th>Leucopenia</th>
<th></th>
<th></th>
<th>Normal count</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Study group</td>
<td>22</td>
<td>44</td>
<td>28</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Illustration 7

Table 7: Absolute neutrophil count profile in cases and control group

<table>
<thead>
<tr>
<th></th>
<th>Suggestive</th>
<th></th>
<th>Not suggestive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Study group</td>
<td>30</td>
<td>60</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Control group</td>
<td>9</td>
<td>36</td>
<td>16</td>
<td>64</td>
</tr>
</tbody>
</table>
Table 8: Band cell- Neutrophil count (BC/NC) ratio profile in cases and controls

<table>
<thead>
<tr>
<th>Ratio&gt;0.2</th>
<th>Ratio&lt;0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Study group (n=50)</td>
<td>34</td>
</tr>
<tr>
<td>Control group (n=25)</td>
<td>8</td>
</tr>
</tbody>
</table>
Illustration 9

Table 9: Sensitivity, specificity and positive predictive accuracy of individual test

<table>
<thead>
<tr>
<th></th>
<th>Total positive test</th>
<th>Positive test with proven sepsis</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>21</td>
<td>20</td>
<td>90.9</td>
<td>96</td>
<td>95.23</td>
</tr>
<tr>
<td>TLC</td>
<td>10</td>
<td>10</td>
<td>45.45</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ANC</td>
<td>23</td>
<td>14</td>
<td>63.63</td>
<td>64</td>
<td>60.68</td>
</tr>
<tr>
<td>BC/NC ratio</td>
<td>24</td>
<td>16</td>
<td>72.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Illustration 10

Graph 1: Sensitivity, specificity and positive predictive accuracy of individual test.
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