



**Comparison of rapid Semi-Quantitative card test against
Immunoturbidimetric Quantitative test for determination of C -
reactive protein levels in Neonatal Sepsis**

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Abstract

The early diagnosis of Neonatal sepsis is important. Because of its various clinical signs and symptoms it is necessary to resort to methods for detecting and treating them as soon as possible. C-reactive protein (CRP) is acute phase protein, widely used in the early diagnosis of Neonatal sepsis. Its rapid synthesis, short half-life and rapid decline with recovery, together with an association between greater increases and serious bacterial infections, have made the CRP test popular. This test is often requested to help discriminate viral infections from bacterial infections or monitor the response to antibiotics. Serial CRP measurement is a good practical guide for discontinuing antibiotic therapy in neonates with suspected sepsis. In present study comparison of a rapid point of care semi quantitative card test was done with quantitative test for measurement of CRP in fifty suspected cases of Neonatal sepsis. Semi quantitative card test is based on the principle of flow through immunochromatography working with whole blood as a samples and hence offer immediate test results where as quantitative test is based on the principle of immunoturbidimetry compatible with serum/plasma as a sample . We have observed very high correlation between two methods in normal as well as abnormal samples. We have also observed that semi quantitative rapid test offer ease of use & interpretation and hence ideal for the physician office laboratory and/or point of care testing.

Key-Words: Neonatal sepsis, C- reactive protein, Immunoassay

Introduction

Neonatal sepsis (NNS) is terms that have been used to describe the systemic response to infection and/or isolation of bacteria from the blood stream in the first 28 days of life. Neonatal sepsis is a fatal disease. Globally, sepsis accounts for 26% of all neonatal deaths¹ with 98% of these deaths occurring in developing countries². Unfortunately despite the availability of effective antibiotics, early diagnosis represents a major challenge because of the non-specific nature of signs and symptoms³⁻⁵ and non-availability of standard microbial culture results in the first 48 hours⁶. This may result in the treatment of as many as 30 uninfected neonates for everyone who is eventually diagnosed to be infected⁷⁻⁹. Blood culture is the gold standard for definitive diagnosis but it takes at least 48 hours by which time the infection may have progressed with important consequences on the morbidity and mortality of the neonate⁸.

There is an abundance of studies evaluating laboratory markers in the diagnosis of neonatal sepsis. Despite the promising results for some diagnostic markers, none of them can consistently diagnose 100% of infected cases. The C - reactive protein (CRP) may, help in the early detection of neonatal sepsis while awaiting blood culture results. CRP may also be invaluable in the management of neonatal sepsis in resource poor centers where facilities for blood culture may not be readily available¹⁰.

CRP is the most extensively studied acute-phase reactant so far, its wide availability and its simple, fast & cost-effective determination make it one of the preferred test in many neonatal intensive care units (NICUs)¹¹. It increases as much as a thousand fold within 4 to 6 hours of an inflammatory process¹² make it useful in the early diagnosis of neonatal sepsis. Upon resolution of the inflammation, CRP levels rapidly decline with an elimination half life of 19 hours¹²⁻¹³ which make it preferred marker in determining the duration of antibiotic therapy. Unlike blood culture, CRP level is not affected by prior antibiotic therapy¹⁴

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and hence useful in the situation where neonates may have been given antibiotics before presentation at the hospital. CRP can be assayed quantitatively or qualitatively. The quantitative method is more widely used in developed countries¹³. It provides rapid, highly sensitive and specific results¹⁵ but requires costly analyzer and is more complex and expensive to perform¹³. It is also not possible to perform this assay at point of care testing. The qualitative method provides results within 15 minutes but it is comparatively less specific. It has the advantage of being simple and easier to perform and interpret and as such can be performed at the patients bed side or side laboratory¹⁵⁻¹⁶. It is also less expensive and requires less skill. The qualitative method may therefore, be more feasible in resource poor centers, where there may be no laboratory services for the investigation of neonates with suspected septicemia. In our study we have compared semi quantitative card test with a quantitative method for measurement of CRP in suspected Neonatal sepsis samples.

Material and Methods

The present study was conducted in Surat, Gujarat during January 2013 to February 2013. Leftover samples from hundred suspected neonatal septicemia cases were analysed. Septicemia was assessed with Jaswal's "Sepsis score"¹⁷⁻¹⁸ which include sign and symptoms such as refusal for feed, abdominal distention, vomiting, lethargy, jaundice, poor cry, seizure, diarrhea, apnea, tachypnea, hypothermia, fever and poor capillary refill, if baby had three or more than three of above sign and symptoms, septicemia was suspected.

CRP values were estimated by semi quantitative card test & quantitative immunoturbidimetric test. Semi quantitative INTEX®-CRP Card Test kit was obtained by Intex Diagnostika, Switzerland and Quantitative immunoturbidimetric Turbigold CRP test kit was obtained from Span Diagnostic Ltd, India.

INTEX®-CRP card is an immunochromatographic test for the determination of the CRP concentration in human blood. Test requires 40 µl of 1:80 diluted Whole blood. CRP contained in the sample binds to an anti-CRP antibody immobilized on a permeable membrane at the test region. In subsequent step colloidal gold-coupled monoclonal anti-human-CRP antibody is added to the test field. After a washing step the quantity of the bound colloidal gold can be determined by the resulting color at test region. The intensity of the color is proportional to the content of CRP in the sample. By comparing color intensity of the color with five reference color zone surrounding test region, CRP

value of test sample can be quantified as 10, 25, 50, 100 & 200 mg/L.

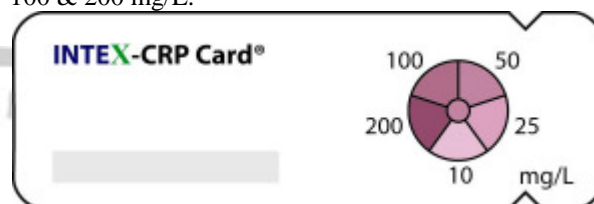


Fig. 1: INTEX®-CRP Card test

Quantitative CRP test is working on the principle of immunoturbidimetry in which Latex particles coated with anti-human C reactive protein (CRP) antibodies agglutinate when mixed with sample containing CRP, resulting into insoluble antigen-antibody complexes. These insoluble complexes increase the turbidity, which is measured at 550 nm. Increase in turbidity is directly proportional to concentration of CRP in the sample.

Results and Discussion

Results 34 out of total samples were within normal limit by both methods. 20 samples were border line positive i.e. between 10-25 mg/L by semi quantitative method, all these samples were in the same range by immunoturbidimetric method. Similarly 22 samples were in moderately positive range i.e., between 25-50 mg/L by semi quantitative method, except 2 sample which was above 50 mg/L all other samples were in the same range by immunoturbidimetric method. CRP results of semi quantitative method in 50 -100 mg/L and 100 to 200 mg/L range were completely correlating with respective range of immunoturbidimetric method.

Overall results of semi quantitative method have shown very high correlation with Quantitative results. Two samples which were not in agreement quantitatively were qualitatively in agreement and hence not having any adverse effect on diagnosis.

We have observed very high correlation between Semi quantitative card test and quantitative immunoturbidimetric test. In our study Semi quantitative card test have shown reliable performance at lower detection level unlike Hilary Valiance and Gihian Lockitch¹⁹ who have found slight tendency to overestimate CRP values by the semi-quantitative method specifically at lower detection level. Our results are in agreement with findings of Petter *et al.*,²⁰ in their study they have concluded that Nycocard - Semi quantitative card test results correlated well with quantitative method and Enrico *et al.*²¹ who have reported excellent correlation of two commercially available rapid bed side test with a validated CRP assay in newborn infants.

CRP card is simple to perform 2-3 minutes per sample test, works with whole blood without the use of expensive equipment. Because of its simplicity in performance and visual result interpretation it can be performed by the less skilled person. CRP card test has several methodological advantages against other commonly used CRP assay one of most important is that it works directly with small volume of whole blood. This makes it especially well suited for pediatric use and decentralised testing. While performing a test mixing of small volume of blood with sample dilution medium lyses blood cells and the cell fragments thus obtained soak through the porous membrane to which the sample is applied. The assay measure CRP concentrations as low as 10 mg/L. CRP Card test is not subject to prozone effects like agglutination and turbidimetric assays being a solid phase immunoassay with sequential addition of reagents. As assay is performed on lysed blood, hemolysis of blood is not interfering in result interpretation. Lipids, bilirubin, and rheumatoid factor should not markedly interfere with the assay because the sample is initially diluted and CRP is subsequently concentrated by the use of solid-phase CRP antibodies. Also sample passes quickly through the porous membrane thereby reducing low-affinity nonspecific binding and minimizing chemical interference. CRP measurements are used for diagnosis and monitoring which require quantitative measurement. This test is less precise than quantitative assay as it gives semi quantitative test results which show high correlation with Quantitative assay and hence still acceptable for decentralised use where quantitative testing facility is not available. In hospital laboratories it might also be used for rapid analyses for samples containing large quantities of interfering substances like bilirubin, lipid, rheumatoid factor etc.

Conclusion

All above advantages makes CRP card test most suited for measurement of CRP in neonates for early diagnosis of sepsis. The main limitation of this study was the small number of samples and the lack of a control group therefore further study and larger multicentre sample would be of value.

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Table 1: Results of Quantitative vs. Semi quantitative method

Quantitative test results (mg/L)	Semi quantitative test results (mg/L)					
	<10	10 - 25	25 -50	50 - 100	100 - 200	Total
<6	34					34
6 - 25		20				20
25 -50			22	2		24
50 - 100				14		14
100 - 200					8	8
Total	34	20	22	16	8	100

Table 2: Agreement of CRP results between Quantitative and Semi-quantitative method

CRP concentration (mg/L)	No. of samples in agreement	No. of samples in disagreement
<10	34	0
10 – 25	20	0
25 – 50	22	0
50 – 100	14	2 (25-50)
100 – 200	8	0
Total	98	2