Point-of-Care-Testing **Edited by: P. Luppa**

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Clinical evaluation of point-of-care testing for wide-range C-reactive protein (wr-CRP) in neonates with suspected sepsis

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Abstract

Background: The aim of this study was to validate a point-of-care C-reactive protein (CRP) test (QuikRead, wide-range [wr] CRP) against standard laboratory testing in neonates with suspected sepsis.
Methods: This was a single-centre prospective cohort study of neonates (n = 91). The main outcome measure was the paired evaluation of the wr-CRP point-of-care test and automated laboratory CRP tests in neonates with suspected sepsis.
Results: There were 126 measured CRP-sample pairs. The mean difference between the laboratory CRP and the wr-CRP point-of-care test values was 0.19 (95% confidence interval [CI]:–1.0–0.65). Pearson's correlation coefficient was 0.94. The area under the receiver operating characteristic (ROC) curve was 0.99 (95% CI: 0.98–1.00). At a QuikRead CRP cut-off of ≥6.2, the sensitivity and specificity were 77% and 100%, respectively.
Conclusions: Point-of-care wr-CRP testing can be used as a screening test in neonates with suspected sepsis. Rapid bed-side diagnostics and minimal blood volume requirements present an attractive alternative to common laboratory CRP testing.

Keywords: C-reactive protein (CRP); neonatal sepsis; point-of-care test; preterm infant.

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Introduction

Early-onset neonatal bacterial infection (infection with an onset within 72 h of birth) is a significant cause of mortality and morbidity in newborn babies. The National Institute for Health and Care Excellence (NICE) recommends that babies with suspected early-onset neonatal infection should be treated as quickly as possible.

C-reactive protein (CRP) is an acute phase reactant. Following the onset of an acute phase response, serum levels rise rapidly and extensively, peaking at 24–48 h. CRP is a valuable marker of infection and can support or refute the early diagnosis of neonatal sepsis.

Standard laboratory CRP testing requires specialised equipment and experienced personnel, and the results are available within 1–2 h at best.

Point-of-care testing (POCT) refers to any diagnostic test administered outside the central laboratory at or near the location of the patient. The aim of POCT is to cut down on any transport/processing delays and result in rapid feedback of the test results to medical decision-makers.

There are now CRP point-of-care tests available. These have many advantages as they can provide results within minutes and can be used by clinicians or nursing staff. They also require smaller aliquots of blood. Blood conservation is an extremely important consideration in neonates to reduce the incidence of iatrogenic anaemia.

The aim of this study was to evaluate the wide-range (wr)-CRP point-of-care test, using a QuikRead instrument, in a neonatal population and compare the results to our standard laboratory Roche/Hitachi Cobas c 701 analyser.

Materials and methods

Study design and setting

This prospective study was conducted in the neonatal intensive care unit (NICU) at the Southmead Hospital in

Bristol over a 10-month period, from June 2016 to March 2017. The study was in the form of a diagnostic test evaluation.

Study protocol

Inclusion criteria

All neonates that required a CRP, either as part of a septic work-up or for the ongoing management of infection, were eligible for the study.

Exclusion criteria

Neonates were excluded if a QuikRead CRP or laboratory CRP result was not available.

Data collection

All health care professionals in the NICU were given training in the use of the QuikRead 101 instrument (Orion Diagnostica Oy, Espoo, Finland) for CRP testing. The patients' hospital numbers were recorded on pre-designed data collection sheets whenever a QuikRead CRP sample was ordered. One dedicated physician collected the data on basic patient demographics and the paired QuikRead and laboratory CRP values.

Blood sampling and laboratory methods

Whenever a laboratory CRP was ordered, CRP concentrations from the same venepuncture or capillary blood sample were also determined using the QuikRead wr-CRP point-of-care test.

Both the QuikRead wr-CRP POCT and reference laboratory CRP tests are quantitative tests that determine the CRP level by immunoturbidimetry. This is when the turbidity of a sample is used to determine the level of an analyte. An assay reagent containing latex particles coated with monoclonal antibodies is added to the human CRP sample. The antigens on the CRP molecules agglutinate with the antibodies forming an immune complex that precipitates increasing the turbidity of the sample.

The QuikRead wr-CRP POCT requires only 0.1 mL whole blood and produces quantitative results with a measuring range of 0.5–180 mg/L for whole blood (the measuring range for plasma/serum samples is 0.5–300 mg/L). The test can produce a CRP result in approximately 3 min. The method utilises a specific instrument (QuikRead 101), capillaries, cuvettes and reagents. After filling the provided capillaries with venous or capillary blood, the specimen is dispensed to a cuvette, containing a 1-mL buffer. The cuvette is then gently shaken to mix the contents. The cuvette is then placed into an instrument well to derive a control measure which takes 40 s. The cuvette is then taken out of the instrument well and the cap containing the latex reagent is depressed, releasing it into the sample. The cuvette is gently remixed and returned to the instrument well where a CRP concentration is measured within 2 min. The final QuikRead wr-CRP POCT is automatically calculated using its wide range of haematocrit correction (15–75%).

In this study, the measurement of the QuikRead wr-CRP levels was performed using 0.1 mL of whole blood that was extracted from the same sample being sent to the laboratory for the reference CRP tests.

The CRP concentration was determined in the Biochemistry Department of our hospital by a particle-enhanced immunoturbidimetric assay using the Roche/Hitachi Cobas c 701 analyser (F. Hoffmann-La Roche Ltd., Basel, Switzerland). A whole blood sample was sent to the laboratory where it was centrifuged, and the resulting plasma/serum was then analysed. A minimum volume of 0.1 mL of plasma/serum was required to run the test; therefore, the recommended whole blood volume was 0.3 mL. The measuring range for the Roche/Hitachi Cobas c 701 analyser for plasma/serum samples is 0.3–350 mg/L.

The laboratory CRP test has a recommended cut-off of ≥5 mg/L. The laboratory CRP readings were performed by trained technicians who were blinded to and not involved in the decision-making process involving the study patients.

Statistical analysis

Data were captured from the data collection sheets into Microsoft Office Excel and analysed using the Stata 14 software.

The basic patient demographics are described as medians with ranges and percentages of the total sample size.

The CRP data are expressed as medians with their respective interquartile ranges.

The degree of linearity between the CRP levels obtained via the two different methods is illustrated in a scatterplot graph with an appropriately fitted regression line.

Two statistical analyses were used to compare the QuikRead CRP values with the laboratory CRP values. These included Pearson's correlation, which was run to determine the relationship between the CRP values, and the Bland-Altman test to assess the absolute differences and the means between the tests.

The sensitivities and specificities were calculated using the laboratory CRP test as the reference test against various cut-off values on the QuikRead CRP test. A prediction of the measurement of likely sepsis was defined as a laboratory CRP of ≥5 mg/L. Using ROC tables, the cut-off value for the QuikRead CRP POCT that represented the most optimal sensitivity and specificity was derived.

Ethical statement

The study protocol was approved by the National Health Service (NHS) England Health Research Authority (HRA). As the study was a quality audit design and was given anonymised data, the committee requested no parental informed consent.

Results

Study population

During the 10-month study period, a total of 91 neonates were tested. The demographic data with the medians and interquartile ranges are given in Table 1. One hundred and forty-seven QuikRead wr-CRP tests were performed. Excluding 21 tests where a paired sample was not available left a total number of 126 samples for analysis. The vast majority of samples (87%) were capillary in origin.

The medians, ranges, and the 25th and 75th percentiles of the CRP levels determined by the laboratory and QuikRead CRP tests are shown in Table 2.

Table 1: Infant characteristics.

Characteristics	All patients (n = 91)
Gestational age	
Median, weeks	38.8
Interquartile range: 25th–75th percentile, weeks	29–40.6
Birthweight	
Median, g	3078
Interquartile range: 25th–75th percentile, g	1410–3754
Gender	
Male, n (%)	52 (57%)
Female, n (%)	39 (43%)
Mode of delivery	
Caesarean section	41 (45%)
Vaginal delivery	50 (55%)

Table 2: Comparison between the QuikRead wr-CRP POCT and the laboratory CRP test.

	QuikRead CRP	Laboratory CRP
Median, mg/L	2.2	3
Measuring range, mg/L	0.5–180 (whole blood)	0.3–350 (plasma/serum)
25th percentile, mg/L	0.5	1
75th percentile, mg/L	14.0	15.5

CRP, C-reactive protein.

Paired haemoglobin and haematocrit levels were also determined in 93 out of 126 samples. The median haemoglobin identified was 153 mg/L and the median haematocrit was 46%.

Evaluation of relationship between standard laboratory testing and QuikRead CRP

Simple (univariate) regression

A scatterplot graph with an appropriately fitted regression line is shown in Figure 1. The adjusted regression coefficient of determination (R^2) was 0.98. This means that 98% of variation in the QuikRead CRP POCT is explained by the laboratory CRP test. The slope coefficient was 0.94 with a 95% confidence interval (CI) of 0.92–0.96. Therefore, when the laboratory CRP increased by 1.0 mg/L, the QuikRead CRP POCT closely correlated by an increase of 0.94 mg/L.

Bland-Altman plot

The absolute differences between the two CRP tests against their means is illustrated in Figure 2 in a Bland-Altman

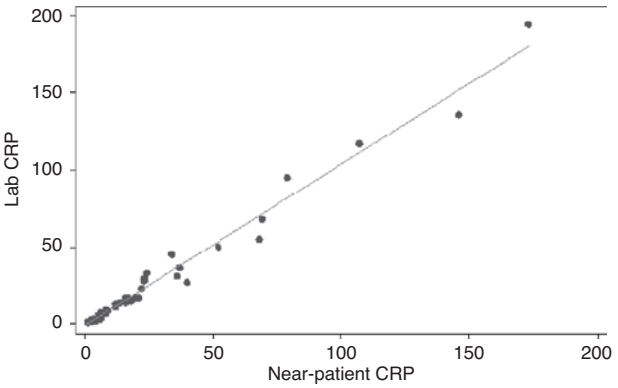

Figure 1: Linear regression analysis between the laboratory CRP test and the QuikRead CRP POCT.

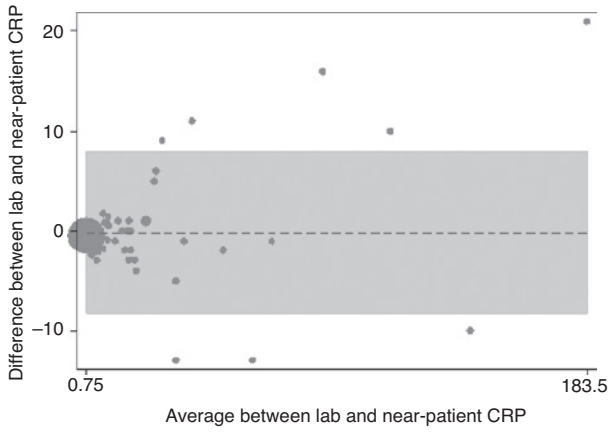

Figure 2: The Bland-Altman plot shows the differences plotted against the averages of the two sets of CRP values, obtained from the QuikRead CRP POCT and laboratory analyser.

plot. This shows there is an increase in variability between the two tests as the QuikRead CRP levels increase. Clinically, a difference between the two tests becomes less relevant as the CRP levels increase. However, a difference between the two tests is relevant when the CRP is low, especially as we use cut-off values to support our diagnosis of sepsis.

ROC curve

In our study, we used a cut-off value of CRP >5 mg/L to define sepsis. This was applied to both the laboratory and QuikRead CRP POCT results. Shown in Figure 3 is an ROC curve which we used to show at what threshold the QuikRead CRP POCT is predictable of a CRP >5 mg/L in the laboratory. We found that if the QuikRead CRP POCT is ≥6.2 mg/L, the specificity is 100% with a sensitivity of 77%. This will still miss some cases, so we found that the optimal cut-off value of the QuikRead CRP POCT with a 100% sensitivity and 94% specificity is ≥3 mg/L. The area under the ROC curve is 0.99 with a 95% CI of 0.98–1.00 and a p-value of 0.0047, which implies that the QuikRead CRP POCT is a good diagnostic test.

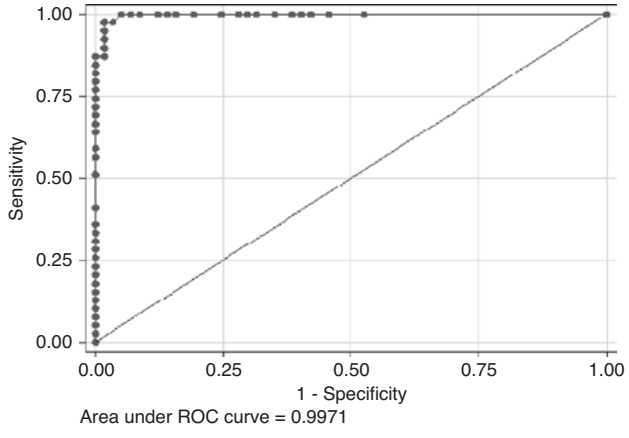

Figure 3: ROC curve for the QuikRead CRP POCT.

Sub-group analysis

We attempted to correct for clustering in the analysis and tested to see if there was an interaction between both of the CRP tests (laboratory and QuikRead), polycythaemia and gestational age.

Polycythaemia (haematocrit >50%)

CRP whole blood POCTs need to be calibrated so that they correlate with the corresponding serum/plasma level. The mean normal range of haematocrit in adults is 40%, whereas haematocrit values can range from 29 to 68% in paediatric samples [1]. Any deviations from a haematocrit of 40% need to be corrected using a haematocrit correction factor. In this study, the point-of-care QuikRead wr-CRP POCT automatically calibrates for haematocrit using its wide range of haematocrit corrections (15–75%). However, we tested for an association between a haematocrit of >50% and CRP values. We found no evidence that the QuikRead CRP and laboratory CRP are modified by a haematocrit of >50% (p-value 0.24).

Gestational age

We also considered whether gestational age is associated with a difference in CRP values. Research has shown that different CRP reference intervals are needed for healthy term and preterm neonates due to the strong positive effect of gestational age on the elevation [2]. We found no evidence that the QuikRead CRP and laboratory CRP are modified by gestational age (p-value 0.26).

Discussion

Early-onset neonatal bacterial infection (infection with an onset within 72 h of birth) is a significant cause of mortality and morbidity in newborn babies. If the diagnosis of neonatal sepsis is delayed, its clinical course can be rapidly fatal. CRP is a valuable marker of neonatal sepsis. Standard laboratory CRP results can take 1–2 h at best to become

available and usually a minimum of 0.3 mL is required for sampling. Bedside CRP testing provides results rapidly and requires less blood sampling, both relevant factors in a neonatal population.

This was a diagnostic test evaluation study in which the point-of-care QuikRead wr-CRP test was compared to our standard laboratory testing in babies with suspected infection. This is the first report of its kind, where this specific POCT has been trialled in this setting.

Our study found that the point-of-care QuikRead CRP results were in excellent agreement with the laboratory CRP values with Pearson's correlation co-efficient of 0.94 (0.92–0.96). An increase in variability was discovered between the two tests as the QuikRead CRP levels increase; however, at low levels (which are arguably more important), there is less variation. An optimal cut-off of ≥3 mg/L with the point-of-care QuikRead wr-CRP POCT is recommended to reflect a laboratory CRP value of ≥5 mg/L. No correlation was found between haematocrit and the gestational age and the QuikRead or laboratory CRP values.

The QuikRead CRP POCT has been validated in the paediatric population. However, most of the studies are based in a primary care or paediatric emergency department setting. In the study by Esposito et al. [3], the QuikRead CRP POCT was compared with standard laboratory testing in children <14 years of age who arrived at their paediatric emergency department with respiratory infection. Studies by Nafar et al. [4, 5] also used the QuikRead CRP POCT in children with suspected bacterial pneumonia and gastroenteritis in the paediatric emergency department. In these studies, the QuikRead CRP POCT was found to be a useful predictor of infection and correlated well with standard laboratory testing.

There are very few studies in the literature that have used point-of-care CRP testing in a predominately neonatal setting. Much of the evidence available is also based on a predecessor version of the technology. Advances have been made that make this testing kit more applicable to the neonatal population. In a study by Diar et al. [6], a predecessor version of the QuikRead CRP POCT was used to analyse a neonatal population in Soweto, South Africa. Their conclusion was that it would be a useful screening test in cases where a clinical diagnosis of sepsis was in doubt. They found that a CRP cut-off level of 16.2 mg/L for the QuikRead CRP POCT minimised the possibility of a false-negative result. The QuikRead CRP POCT used in this study had a CRP detection limit of 8.0–160 mg/L. The median CRP concentrations were 6 mg/L and 9 mg/Lin the first 24 h after birth in septic preterm and term neonates, respectively [7]. One of the strengths of our study is that we used a QuikRead version with a wide measuring range (0.5–180 mg/L for whole blood). This is important in neonates where highly sensitive CRP assays that can reliably measure low CRP values are needed. It also had a wide range of automatic haematocrit correction (15–75%) leading to more reliable results.

There are other studies that have used alternative point-of-care CRP testing in a neonatal setting. Aydin et al. [8] used NycoCard CRP POCT and compared them with laboratory CRP tests in 63 babies. They concluded that the NycoCard CRP POCT is highly predictive. In a study by Zecca et al. [9] where both the Nycocard and QuikRead CRP POCTs were compared to standard laboratory testing, both bedside tests had good specificity (QuikRead 80.5%, NycoCard 83.3%) and sensitivity (QuikRead 97.2%, NycoCard 94.4%). The agreement of measurement with central laboratory values was high for both the bedside tests, without statistically significant differences between the methods. In similar findings to our study, the accuracy of the results of both bedside tests was found to be decreased when CRP concentrations were >100 mg/L.

Laboratory testing leading to phlebotomy losses during the first few weeks of life is one of the main causes for anaemia in extremely low birth weight (ELBW) infants. Point-of-care tests use minimal sample volumes and thus have potential for reducing the incidence of phlebotomy-associated anaemia in neonates. In our study, 0.1 mL of whole blood was required for the POCT compared to 0.3 mL whole blood needed as a minimum for standard laboratory testing. Madan et al. [10] introduced a point-of-care blood gas analyser in their NICU and found that the mean volume of red blood cell transfusions in ELBW infants decreased by 43% in the first 2 weeks of life.

Our study has some limitations. It is a small study of only 91 neonates and 126 paired samples. We have included infants of variable gestation and weight who had varying methods of venepuncture (mostly capillary) to determine the CRP values. We have attempted to correct for this clustering of data and found no interaction with gestational age or haematocrit values. However, there is limited power to look at the subgroup analysis, and some questions remain unanswered. We also defined a cut-off CRP level based on the data collected but have not yet tested this in practice. Therefore, a follow-up study is required to determine if this cut-off value is reliable.

The NICE recognises the benefits of point-of-care CRP testing and has already published guidance on its use. When managing pneumonia in adults based within a primary care setting, the NICE recommend that point-of-care CRP testing should be considered if a diagnosis is unclear after clinical assessment, and that antibiotics should be prescribed based on the result [11]. In September

2016, the NICE published a MedTech innovation briefing on the QuikRead CRP POCT for CRP testing in primary care [12]. Evidence from several studies (n = 4874) showed that the QuikRead CRP POCT performs with similar accuracy to the standard laboratory CRP test in detecting pneumonia and results in reduced antibiotic prescribing rates compared with standard care. The NICE believes that "using this technology could contribute to fulfilling antibiotic stewardship programmes".

The innovative aspects of using CRP POCT in the NICU are that it is quick, reliable and only requires 0.1 mL of blood. In addition to reducing the risk of iatrogenic anaemia secondary to blood sampling, serial point-of-care CRP measurements are likely to assist with earlier discontinuation of antibiotic therapy in support of antibiotic stewardship.

Conclusions

The point-of-care QuikRead wr-CRP test provides reliable results in a neonatal population when compared to standard laboratory testing and might be used as a bedside tool to aid decisions in the management of neonatal infection.

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