Usefulness of a semi-quantitative procalcitonin test kit for early diagnosis of neonatal sepsis

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ABSTRACT

Introduction: This study was designed to determine the sensitivity and specificity of a semi-quantitative procalcitonin (PCT) test kit for the diagnosis of neonatal sepsis.

Methods: Infants admitted to the neonatal intensive care unit with signs suggestive of sepsis were recruited into the study. Prior to commencement on antibiotics, the following investigations were carried out on each of these infants: blood culture and sensitivity, PCT semi-quantitation and C-reactive protein (CRP) estimation. Infants already on antibiotics, or who developed signs of sepsis within 72 hours of discontinuation of antibiotics, were excluded from the study.

Results: Of the 87 infants recruited, 18 (20.7 percent) were confirmed to have sepsis based on positive blood culture results. At a PCT cut-off level of greater than or equal to 2 ng/ml, the sensitivity of the PCT-Q kit in detecting neonatal sepsis at the onset of symptoms was 88.9 percent and its specificity was 65.2 percent. The sensitivity of CRP for diagnosis of sepsis was 55.6 percent and its specificity was 89.9 percent.

Conclusion: The semi-quantitative PCT test kit is of moderate sensitivity but poor specificity for early diagnosis of neonatal sepsis. A negative PCT test result may help to "rule out", while a raised CRP result helps to "rule in", the possibility of sepsis.

Keywords: neonatal sepsis, procalcitonin, semi-quantitative test kit

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INTRODUCTION

Clinical manifestation of sepsis in newborn infants is usually nonspecific. Because of the high morbidity and mortality associated with neonatal sepsis,[1,2] antibiotic therapy is commenced soon after the onset of symptoms before diagnosis is confirmed by blood culture. Furthermore, infants with sepsis may be under-diagnosed, if the volume of blood obtained for blood culture was too small (less than 0.5 ml). As a result, over-treatment with antibiotics is a common practice in the neonatal intensive care unit (NICU).

Procalcitonin (PCT) is a 116-amino acid protein with a sequence identical to that of the hormone, calcitonin.[3] Under normal metabolic conditions, hormonally-active calcitonin is produced and secreted in the C-cell of the thyroid gland after specific intracellular proteolytic procession of the pro-hormone PCT. However, in severe bacterial infections and sepsis, macrophages and monocytic cells of various organs, such as the liver, are believed to be involved in the synthesis and release of PCT in response to bacterial infections.[4,5] Several studies have reported on the usefulness of quantitative measurement of PCT for early diagnosis of sepsis in newborns,[6-8] as neonates with viral infection, bacterial colonisation or distress due to other causes, had normal or only slightly elevated PCT levels.[6]

Recently, a semi-quantitative PCT test kit has become commercially available. This kit is easy to use at the bedside, providing rapid results and requiring a very small amount of blood.[9] Studies in adults showed that this test kit has a high sensitivity and specificity of diagnosing bacterial sepsis in patients with PCT levels of ≥ 2.0 ng/ml when compared with quantitative measurement of PCT.[9] At the time of initiation of the present study, no study had been reported on the usefulness of this semi-quantitative PCT test kit for the diagnosis of sepsis in newborns. The objectives of this study were to determine the percentage of neonates with confirmed sepsis and who had raised PCT level ≥ 2.0 ng/ml measured by the semi-quantitative PCT-Q test, and the sensitivity and specificity of this test kit for the diagnosis of sepsis at the onset of symptoms in newborn infants.

METHODS

This was a prospective observational study carried out over a 24-month period (January 1, 2005 to December 31, 2006) in the NICU of Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur. The study protocol was approved by the institutional Scientific and Ethics Committees. The inclusion criteria were infants admitted to this NICU

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with signs suggestive of sepsis, or who developed signs of sepsis while in the ward. The exclusion criteria were infants on antibiotics or developed signs of sepsis within 72 hours of discontinuation of antibiotics.

Upon identification of eligible infants, written parental consent was obtained. Specimens of blood were obtained from each infant for the following investigations: full blood count, blood culture and sensitivity (with blood volume 0.5–1.0 ml), PCT semi-quantitative measurement and C-reactive protein (CRP). Chest radiograph, cerebrospinal fluid culture, tracheal aspirate culture and urine culture were done whenever clinically indicated. Antibiotics were commenced after the blood specimens were collected. The decision to stop antibiotics was based on clinical features and blood culture results.

Specimens of blood (of at least 0.5 ml) were obtained from each infant by a sterile technique and were inoculated into commercially-prepared BD Bactec Peds Aerobic/F vials (Becton Dickenson, Shannon Country Clare, Ireland) at the bedside. The inoculated vials were transported to the microbiological laboratory and inserted into a BACTECT Fluorescent series instrument, BACTECT 9240 (Becton Dickenson, Shannon Country Clare, Ireland) for incubation and periodic reading. Positive cultures were detected by chemical sensors sensitive to increases in carbon dioxide produced by growth of the organisms. The organisms were then identified based on gram staining and growth on agar media.

The test kit PCT-Q (B.R.A.H.M.S. Diagnostica GmbH, Berlin, Germany) is based on the immuno-chromatographic principles for semi-quantitative determination of PCT.[3] It uses a monoclonal mouse anti-catacalcin antibody conjugated with colloidal gold (tracer) and a polyclonal sheep anticalcitonin antibody (solid phase). The test procedure is carried out on a non-haemolysed blood sample that was previously centrifuged, and 200 µL serum is pipetted into the round cavity of the test trip. The tracer binds to the PCT in the sample and a marked antigen antibody complex is formed. This complex moves by means of capillarity through the test systems, and in the process, passes through the area containing the test band. Here, the marked antigen antibody complex binds to the fixed anticalcitonin antibodies and forms a sandwich complex. At a PCT concentration ≥ 0.5 ng/ml, this sandwich complex can be seen as a reddish band.

The colour intensity of the band is directly proportional to the PCT concentration of the sample and it is related to different PCT concentration ranges (≥ 0.5 ng/ml, ≥ 2.0 ng/ml, ≥ 10 ng/ml) with the help of a reference card. Non-bound tracer diffuses into the control band zone, where it is fixed and produces an intensely red colour control band. The functional ability of the test system is checked by means of this control band. After an incubation period of 30 minutes, the results of the test are read and the PCT concentration ranges are determined by comparing the colour intensity of the band with colour blocks of the reference card. Lipids and bilirubin have no effect on the measured result. During the study, the blood samples of all infants were tested within four hours of collection to ensure accuracy.

Serum CRP was measured in the laboratory by particle enhanced immunoturbidimetric methods using the COBAS INTEGRA 800 machine (Roche, Basel, Switzerland) according to the manufacturer's specifications. The inter-assay coefficient of variation (CV) for human serum was 2.9 %, and the intra-assay CV was 1.0%. During the study, a diagnosis of sepsis was made in any symptomatic infants with positive blood culture results. Term infants were those with gestation of ≥ 37 completed weeks, and preterm infants were those with gestation < 37 weeks. PCT-Q level was considered to be raised when it was ≥ 2 ng/ml. Normal CRP level was defined according to age of infants: day 1 to day 4: < 1.5 mg/ml; more than day 4 of age: < 1.0 mg/ml. CRP level was defined to be raised when it exceeded the normal levels at the corresponding age stated above. A minimum sample size of 61 infants was required in order to achieve the lowest acceptable sensitivity (or specificity) of 80% with a confidence intervals of 90% and a target disorder of 30%.[10]

The Statistical Package for Social Sciences for Windows version 12.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The chi-square test (or Fisher's test for expected value of < 5) was used for analysis of categorical variables. The Student's unpaired *t*-test was used for analysis of continuous variables with a normal distribution, and the Mann-Whitney U-test was used for those with a skewed distribution; p-values of < 0.05 were considered statistically significant. Using blood culture results as the gold standard, the sensitivity, specificity, positive predictive values and negative predictive values of the PCT-Q and CRP for diagnosing sepsis were calculated. The sensitivity of a test was defined as the proportion of infants with sepsis and were correctly identified by the test. The specificity of the test was defined as the proportion of infants without sepsis and were correctly identified by the test. The positive predictive value of a test was defined as the proportion of infants with positive test results and who had sepsis. The negative predictive value of a test and was defined as the proportion of infants with negative test results and who did not have sepsis.

RESULTS

During the study period, 87 symptomatic infants underwent blood culture, PCT-Q test and estimation of CRP levels. 18 (20.7%) of them were confirmed to have sepsis (Table I). The majority (66.7% or 12/18) had gram-positive sepsis

Table I. Relationship between types of bacteria and procalcitonin and C-reactive protein levels in 18 infants with confirmed sepsis.

Case no.	Types of organisms	PCT-Q (ng/ml)	CRP (mg/ml)	Age when diagnosed (days)
1	GBS	≥ 10	0.04	1
2	CONS	≥ 10	0.52	8
3	*Acinetobacter* spp.	≥ 0.5	0.02	21
4	CONS	≥ 2	0.1	7
5	*Staphylococcus aureus*	≥ 2	23.92	19
6	CONS	≥ 10	2.48	41
7	CONS	≥ 2	0.65	10
8	*Enterobacter* spp.	≥ 0.5	0.65	15
9	CONS	≥ 10	3.37	6
10	CONS	≥ 10	0.18	5
11	*Acinetobacter* spp.	≥ 2	0.58	3
12	*Klebsiella pneumoniae*	≥ 10	0.32	28
13	CONS	≥ 10	2.83	20
14	*Staphylococcus aureus*	≥ 10	2.95	9
15	CONS	≥ 10	2.93	7
16	*Streptococcus viridans*	≥ 10	5.50	14
17	*Serratia* spp.	≥ 10	9.44	27
18	*Klebsiella* spp.	≥ 10	16.79	17

GBS: group B haemolytic *Streptococcus;* CONS: coagulase-negative *Staphylococcus*

and 33.3% (6/18) had gram-negative sepsis. All infants with gram-positive sepsis and 66.7% (4/6) of infants with gram-negative sepsis had raised PCT levels ≥ 2 mg/ml. Two infants with gram-negative sepsis had low PCT levels of ≤ 0.5 mg/ml. The age of onset of sepsis ranged from day 1 to day 54. Except for one infant, who had group B haemolytic streptococcal sepsis presented on the first day of life, all others presented at more than 48 hours of age. The two infants with gram-negative sepsis and low PCT levels developed sepsis after the second week of life. Only 55.6% (10/18) of the infants with sepsis had raised CPR levels. Most (75%) of the infants with sepsis had PCT levels ≥ 10 mg/ml regardless of the types of organisms they were infected with.

There was no significant difference in the ethnic distribution between infants with and without sepsis, irrespective of their age of onset of presentation (Table II). All study infants, except one without sepsis, survived at discharge. Infants with sepsis had significantly lower birth weight ($p = 0.02$) and gestational age ($p = 0.02$) than those without sepsis. The age of onset of symptoms presented significantly later among infants with sepsis ($p < 0.00001$). A significantly higher proportion ($n = 43$ or 62.3%) of infants without sepsis were symptomatic during the first two days of life, than those with sepsis ($n = 1$, or 5.6%) ($p < 0.0001$). A significantly higher proportion of infants with sepsis had raised PCT level ≥ 2 ng/ml (88.9% vs. 34.8%)($p < 0.001$) and raised CPR levels (55.6% vs. 10.1%)($p < 0.001$) than those without sepsis. The sensitivity of the PCT-Q in detecting sepsis at any age of presentation was 88.9% and its specificity was 65.2 %. The positive predictive value of PCT-Q was 40.0%, and its negative predictive value was 95.7%. The sensitivity of raised CRP for prediction of sepsis was 55.6% and its specificity was 89.9%. The positive predictive value of raised CRP was 58.8% and its negative predictive value was 88.6%.

Among infants who presented with symptoms after 48 hours of age, there was no significant difference in the ethnic distribution, birth weight, gestational age and age of onset of symptoms between infants with ($n = 17$) and without ($n = 26$) sepsis ($p > 0.05$). However, a significantly higher proportion of infants with sepsis after 48 hours of age (15/17 or 88.2%) had raised PCT levels ≥ 2 ng/ml and raised CRP levels (10/17 or 58.8%) than those without sepsis (PCT: 6/26 or 23.1%; CRP: 2/26 or 7.7%) ($p < 0.0001$, $p < 0.0001$, respectively). The sensitivity of the PCT-Q in detecting sepsis after 48 hours of age was 88.2%, its specificity 76.9%, its positive predictive value was 71.4% and its negative predictive value was 90.9%. The sensitivity of CRP for predicting sepsis after 48 hours of life was 58.8%, its specificity was 92.3%, its positive predictive value was 83.3% and its negative predictive value was 77.4%.

The majority (60/87) of the infants recruited were preterm, and 25% (15/60) of this latter group had sepsis. When compared with preterm infants without sepsis, a significantly higher proportion of preterms with sepsis

Table II. Comparison of clinical and laboratory variables between infants with and without microbiologically-proven sepsis.

Variables	Confirmed sepsis (n =18)	No sepsis (n = 69)	p-value
Race (%)			
Malay	7 (38.9)	45 (65.2)	
Chinese	9 (50)	20 (29.0)	0.26
Indian	1 (5.6)	2 (2.9)	
Others	1 (5.6)	2 (2.9)	
Birth weight (g)			
Median	1,060	2,100	0.02*
50% confidence interval	830–1,606	1,205–2,958	
Range	690–3,400	535–4,680	
Gestation (weeks)			
Median	30	34	0.02*
50% confidence interval	28–34	29–38	
Range	25–40	24–41	
Age at onset of symptoms (days)			
Median	12.5	1.0	< 0.0001*
50% confidence interval	6.8–22.5	1.0–7.8	
Range	1.0–54.0	1.0–103	
PCT levels (ng/ml) (%)			
< 0.5	2 (11.1)	28 (40.6)	
≥ 0.5	0 (0)	17 (24.6)	< 0.0001*
≥ 2.0	3 (16.7)	7 (10.1)	
≥ 10	13 (72.2)	17 (24.6)	
Raised CRP levels (%)	10 (55.6)	7 (10.1)	< 0.0001*
Alive at discharge (%)	18 (100)	68 (98.6)	1.0

* statistical significance

had raised PCT (93.3% sepsis vs. 28.9% no sepsis; p < 0.0001) and raised CRP (60% sepsis vs. 6.7% no sepsis; p < 0.0001). Only 11.1% (3/27) of term infants recruited had sepsis. When compared with term infants without sepsis, the proportions of term infants with sepsis who had raised PCT (66.7% sepsis vs. 45.8% no sepsis; p = 0.6) and raised CRP levels (33.3% sepsis vs. 16.7% no sepsis; p = 0.5) were not significantly different.

DISCUSSION

To minimise the possibility of under-diagnosis of sepsis based on blood culture during the present study, a minimum of at least 0.5 ml of blood was obtained from each infant for blood culture. Falsely negative results could occur when the volume of blood specimen inoculated into a culture bottle was less than 0.5 ml.[11,12] Furthermore, infants receiving antibiotic therapy within the previous 72 hours were excluded from the study as partially-treated sepsis could be associated with decreasing levels of PCT.[7] Although the PCT-Q semi-quantitative test kit could detect PCT level at different ranges, the cut-off level of ≥ 2 ng/ml (recommended by the manufacturer) for diagnosing sepsis was based on studies in neonates,[8] children[13] and adults.[9] The present study confirmed the findings of other investigators that PCT was more sensitive than CRP in the detection of neonatal sepsis,[7,8] as the PCT level rose earlier than the CRP level during sepsis.[5] When compared with findings in adult patients, the sensitivity of the semi-quantitative PCT-Q kit for diagnosing sepsis in neonates at a cut-off level of ≥ 2 ng/ml was similar (88.9% in neonates based on the present study vs. 89.6% in adults),[9] although the specificity was much lower (65.2% in neonates based on the present study vs. 91.5% in adults).[9]

In a study on the quantitative measurement of PCT in 177 healthy term and preterm infants of gestation more than 32 weeks at the ages of 1–15 days, Gendrel et al reported a mean PCT level (< 0.7 ng/ml) which is not significantly different from those of healthy children[6] and adults.[3] However, a study by Chiesa et al on serial quantitative measurements of PCT in normal term infants showed that the levels of PCT were low at birth (with a predicted upper limit of 0.7 ng/ml), raised to a level of 2.0 ng/ml during the first six hours of life, peaked between 21–30 hours of age to a level of 21.0 ng/ml, and gradually decreased to 2.0 ng/ml by 48 hours of life. Based on a cut-off level at the 95th percentile of normal range of PCT level at different ages, Chiesa et al reported that the sensitivity of diagnosing sepsis in neonates during the first 48 hours of life as 85.7%. In the same study, the sensitivity of detecting late onset sepsis at an age after 48 hours of life was 100%.[8]

In the present study, the high proportion of false positive results (and therefore low specificity) based on the cut-off level of ≥ 2 ng/ml of the PCT-Q kits could be partially explained by the normal progressive rise of PCT during the first 48 hours of life for both term[8] and preterm[14] infants. One possible explanation for the decrease in false positive results after the first two days of age, as suggested by the improvement of specificity of the kit from 65.2% to 76.9%, could be due to the age-specific changes in PCT levels. However, unlike the findings of Chiesa et al,[8] the sensitivity of the PCT-Q kit did not improve in infants with late-onset (after two days of age) sepsis in the present study. The results of our study did not show prematurity to be associated with lower sensitivity of the PCT-Q kit. As the number of term infants recruited were very small, it was not certain whether the sensitivity of the PCT-Q kit for early diagnosis of sepsis would be better in this group of infants.

During the present study, the PCT-Q test detected all infants with gram-positive sepsis, but not all infants with gram-negative sepsis. The latter group of infants accounted for all the false negative results despite the fact that their sepsis occurred much later, after the second week of life (Table I). It was not certain what underlying mechanisms were responsible for this difference, as no other studies had reported on this observation previously. Further studies are required to confirm this phenomenon and to determine the underlying mechanisms.

The results of this study showed that the sensitivity of the PCT-Q kit was moderate, but its specificity was poor for early diagnosis of neonatal sepsis. When used alone, it was not a useful tool for helping doctors to decide whether antibiotics should be started or withheld at the onset of symptoms in newborns, while awaiting results of the blood culture. Delay in commencing antibiotic therapy in an infant with sepsis based on a falsely negative PCT-Q kit result could result in serious consequences. Over-treatment of neonates with antibiotics based on false positive results will promote the emergence of multi-resistant bacteria in the NICU. However, when used together with CRP, a negative PCT test result may help to "rule out", while a raised CRP result helps to "rule in", the possibility of sepsis, particularly of the late onset type. Based on the results of the present study, we recommend that commencement of antibiotics in newborn infants should still be based on clinical features, rather than on PCT-Q results alone.

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