VOLUME-7, ISSUE-1, JANUARY-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pathology

PERFORMANCE OF PROCALCITONIN (PCT) TESTED IN AN AFRICAN PAEDIATRIC INTENSIVE CARE SETTING.

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ABSTRACT

Introduction: It was puzzling for clinicians to get normal CRP reports for patients who displayed clinical features of sepsis. The aim of the study was to test the performance of PCT against traditional markers of sepsis like and white cell counts in a paediatric intensive care setting.

Methods: Data from 75 neonates and 57, > 28 days old patients that had full laboratory results for the investigation of possible sepsis was analysed. Comparative descriptive statistics were used to highlight correlations of biomarkers in sepsis and measure diagnostic accuracy.

Results: The overall correlation of CRP and PCT was weak (r = 0.24) but better (moderate; r = 0.45) at age > 3 days, but very weak at < 2 days. The correlation was also better (moderate; r = 0.53) at PCT ≤ 0.5 µg/L but weak at PCT ≤ 0.27 µg/L. PCT levels correlated with one or with all markers of sepsis and in some instances, was the only pointer of sepsis. Using blood culture as a gold standard, CRP showed more sensitivity compared to PCT (90.5% vs 61.9%), while PCT had more specificity than CRP (27.8% vs 13.9%).

Conclusion: There is a weak correlation between CRP and PCT as markers of sepsis in neonates. CRP is more sensitive but less specific than PCT. Therefore, the use of both tests in neonates when investigating sepsis is recommended.

KEYWORDS : C - reactive protein (CRP), Procalcitonin (PCT), White cell count (WCC)

Introduction

Bacterial infections are common causes of morbidity and mortality in critical care especially in paediatric/neonatal periods. This may be accompanied by clinical signs such as changes in body temperature, tachycardia, changes in white cell counts (WCC) and blood cultures. [1,2] In our experience, clinical signs alone are often inadequate for diagnosis of infection especially in neonates as these signs may mimic other pathologies; and the age-related reference range often compound the difficult to make diagnosis. Sepsis is also a challenge with clinical signs and most laboratory findings occurring when multiple organ failure has occurred. [3] Infections and sepsis are associated with clinical and laboratory signs of e.g. body temperature changes, alterations in C-reactive protein (CRP) levels and leucocytosis as well as tachycardia but these have low discrimination in diagnosis. [4] Further it has been reported that bacteriological positivity of infection may not develop at the same time with clinical signs of sepsis. [5] Changes in WCC as markers of infection cannot also be entirely relied upon, as these can be due to other conditions that cause inflammation but without infection. At Chris Hani Baragwanath Academic Hospital, , positive blood cultures are used to assist in diagnosis of infections; however further sero-biochemical typing and sensitivity testing are sometimes required, but this may take longer. Sterility can be a challenge and we have experienced incidences of contamination, thus making diagnosis of infection based on cultures alone difficult.

Lack of specificity of markers of early infection has been cited as responsible at least in part for withholding or delaying or un-necessary antimicrobial treatment in critical ill patients. [6] Procalcitonin (PCT), a possible marker of systemic inflammatory response to infection, is an acute phase protein that is elevated in blood in sepsis. Levels of PCT have been reportedly elevated in a thyroidectomized patient [4, 7] thus it is possible that PCT associated with sepsis is produced elsewhere besides the thyroid gland [5] e.g. mononuclear leucocytes. [8] Several studies have indicated that PCT is a good marker of sepsis. [6, 9, 10, 11, 12] CRP is increased during bacterial sepsis but is not diagnostic as it can be increased by various causes of inflammation, whereas PCT appears to be increased mainly by bacterial sepsis. [2] PCT levels in blood are increased in bacterial infections but not in viral infections [3] and high PCT levels have been reported to correlate with the severity of microbial invasion. [14]

At Chris Hani Baragwanath Academic Hospital measurement of CRP level assists with diagnosis of sepsis. . A pilot audit conducted at three South African hospitals that examined CRP results of the neonates, observed that more than 70% of the results were normal. However, some neonates with normal CRP results were later diagnosed with sepsis. It is therefore, important to have a biological marker for early detection of sepsis and PCT shows some features for such a marker. A study at the Paediatric University Hospital, at Lodz, in Poland looked at PCT in the early diagnosis of nosocomial sepsis in preterm neonates and reported PCT to be useful in diagnosis of sepsis. [15] Early identification of infections is a challenge and the consensus in our setting is not to administer antibiotics for every suspected infection because of bacterial resistance. Therefore, it is important to have a marker more specific than the traditional markers of infection. Many institutions in the southern African region rely on the combination of the traditional markers; body temperature, tachycardia, WCC and positive cultures. Factors contributing to this are not clear but possibly include unclear and inadequate information on biomarkers and variable reference ranges of PCT in the neonates as well as perhaps prohibitive costs of the tests.

This study therefore, investigated the use of PCT and CRP compared to blood cultures used in diagnosing sepsis in neonates. Primarily the study aimed to describe the correlation between CRP, PCT and WCC as markers of sepsis in neonates. The secondary aim was to compare the diagnostic accuracy of PCT and CRP using blood culture as a gold standard.

Methods

A retrospective study was carried out on randomly selected samples sent to pathology at the Chris Hani Baragwanath, Mowbray and Red Cross Hospitals in South Africa. The study was approved by the University of Witwatersrand Ethics Committee and the DISA Data manager, which is the National Health Laboratory Services' Laboratory Information System, was used for the identification of samples. Between 2010 and 2011, data from 75 neonatal and 57, > 28 days old that had full laboratory results for sepsis diagnosis was analysed: PCT, CRP, WCC and Blood Culture were selected. The patients were from the neonatal intensive care unit (NICU) and paediatric intensive care unit (ICU). The DISA data manager was utilised to select patients with requests for other sepsis screening tests such WCC and blood cultures. Subjects with both PCT and microbiology assays conducted within 48 hours of each other were included.

Measurements: Measurements of PCT (Immuno-luminometric) on the BRAHAMS PCT LIA by B·R·A·H·M·S GmbH, Neuendorfstrasse 25, D-16761 Hennigsdorf in Germany and CRP (Immunoturbidmetric) on Roche Hitachi 917 by Hoffmann-La Roche AG a Swiss global health-care company were carried out according to the manufacturer's instructions. Sensitivity and specificity of CRP and PCT as markers of sepsis were evaluated using positive blood cultures as the gold standard test for sepsis.

Data analysis: The reference values used in our laboratory are: PCT: < 0.05 µg/L (normal), 0.05 - 2 µg/L (grey area that requires repeat after 6 - 24 hours) >2 µg/L (suggestive of an infectious process with systemic consequences) and > 10 µg/L (sepsis). The CRP reference range is 0 - 10 mg/L. Comparative descriptive statistics was used to highlight correlations of biomarkers in sepsis.

Interpretations: results of correlation coefficient (r) are classified or interpreted as very weak (r < 0.20), weak (r = 0.20 – 0.39), moderate (r = 0.40 – 0.69), strong (r = 0.70 – 0.89), or very strong (r ≥ 0.90).[16] Inclusion factors were availability of microbiology results and patients not on antibiotics. Concordance evaluation was performed and in this study was defined as concomitant abnormality of both CRP and PCT results. Diagnostic test accuracy analysis, comparing CRP and PCT to blood culture in the 57 neonates with full data was carried out in Stata 12. Measures reported are sensitivity, specificity, likelihood ratios, positive and negative predictive values. 95% confidence intervals were also reported for each measure.

Results

Correlation between PCT, CRP and WCC

The characteristics of the cohort is summarised in Table 1 below

Table 1: Comparative descriptive statistics of sepsis versus non-sepsis subgroups

	Age (months)		PCT (µg/L)		CRP (mg/L)	
	Sepsis	Non-sepsis	Sepsis	Non-sepsis	Sepsis	Non-sepsis
Mean	18.43	27.71	26.14	12.57	81.35	79.48
Median	17.00	31.00	1.40	1.68	73.70	44.70
SD	14.76	23.44	108.60	31.19	58.34	99.90
Maximum	52.00	77.00	500.00	139.20	223.20	340.80
Minimum	1.00	1.00	0.11	0.11	1.00	1.00

Low levels of PCT and CRP were observed in both patients with sepsis and non-sepsis patients while high levels of PCT and CRP re observed in the positive subgroup as shown in Table 2 below. Concordance was higher at 3 days or older in both positive and negative for sepsis groups and the highest concordance for PCT and CRP was seen in the non-sepsis group at 3 days or above (Table 2).

Table 2: Concordance level between CRP and PCT in sepsis versus non-sepsis subgroups

Group		N	PCT > 0.5 µg/L	CRP > 10 mg/L	CRP concordance
Positive	≤2days	4	0	3	0
	≥3days	17	13	16	81
Negative	≤2days	20	14	20	70
	≥3days	16	12	11	92

In evaluating the variation of CRP in subgroups of normal versus abnormal levels of PCT, it was observed that on average, the levels of CRP were higher at < 2 days compared to > 3 days as shown table 2 above. The overall correlation of CRP and PCT was weak (r = 0.24) but it was better (moderate; r = 0.45) at age > 3 days, but very weak at age < 2 days. Further the correlation was also better (moderate; r = 0.53) at PCT ≤ 0.5µg/L but weak at PCT ≤ 0.27µg/L.

The reference values for PCT: < 0.05 µg/L (normal) with values between 0.05 - 2 µg/L falling in the grey area that requires repeat testing after 6 - 24 hours) >2 µg/L (suggestive of an infectious process with systemic consequences) and > 10 µg/L (sepsis), for CRP the reference range is 0 - 10 mg/L and WCC is 9 – 37 x 10^9/L. Table 3 shows that PCT and CRP progressively increases with age from neonates to patients over 3 years old while WCC appear not changed in the three age groups.

Table 3: Descriptive data of CRP and PCT levels and WCC in the various age groups

	>3 years			1 month – 3 years			Neonates		
	CRP	PCT	WCC	CRP	PCT	WCC	CRP	PCT	WCC
Mean	220	232	12	102	122	10	18	14	11
SD	118	146	8	101	148	8	48	30	7
Median	204	193	10	74	69	10	2	1	11
Min	1.1	0. 3	0.1	0.2	0.1	0.1	0.1	0.1	1.8
Max	501	500	44	407	500	53	343	172.31	44

Keys: CRP in mg/L; PCT in µg/L; WCC in 10^9/L

In the neonates, the three indices show no correlation (r < 0.01) with one another. Over one month of age, there are observable weak to moderate correlations as shown in Table 4 below

Table 4: Correlations of CRP, PCT and WCC in various age groups

	CRP & PCT	CRP & WCC	PCT & WCC
Neonates	0.01	0.14	-0.06
Leukopenia	0.04	0.24	0.01
Normopenia	0.08	0.62	-0.12
Sepsis	0.69	0.38	-0.13
1 month – 3 years	0.75	-0.23	-0.25
1 - 3 months	0.20	-0.03	0.19
> 3 month - 1 year	0.70	0.05	0.15
> 1 year - 3 years	0.64	-0.18	-0.36
> 3 years	0.28	-0.02	-0.18

Comparison of diagnostic accuracy of CRP and PCT

The diagnostic accuracy for both PCT and CRP, compared to blood culture were analysed and the characteristics are presented in Table5 below. The prevalence of sepsis in the sample was 36.8%. Using the cutoff points described above, CRP sensitivity was higher than that of PCT (90.5% vs 61.9%). This makes CRP highly useful in ruling out sepsis, as negative result is most likely to show absence of sepsis. However, in terms of specificity, PCT performed better compared to CRP (27.8% vs 13.9%). From this, it can be deduced that PCT is more accurate in determining presence of sepsis, although both tests showed really low levels of specificity in the analysis.

Table 5: Diagnostic accuracy of CRP and PCT compared to Blood Culture

Test	Sensitivity (%) (95% CI)	Specificity (%) (95%CI)	Positive likelihood Ratio (95%CI)	Negative Likelihood Ratio (95%CI)	Positive predictive value (%) (95%CI)	Negative predictive value (%) (95%CI)
CRP	90.5 (69.6-98.8)	13.9 (4.7-29.5)	1.05 (0.87-1.27)	0.69 (0.15-3.23)	38 (24.7-52.8)	71.4 (29.0-96.3)
PCT	61.9 38.4-81.9)	27.8 (14.2-45.2)	0.86 (0.58-1.27)	1.37 0.64-2.93)	33.3 (19.1-50.2)	55.6 (30.8-78.5)

Sepsis prevalence was 36.8%. Blood culture is the gold standard for sepsis.

Discussion

This study looked at CRP, PCT and WCC as markers of infections in paediatrics and the results show relatively low concordance of the measures and in the sepsis group by comparison with non-sepsis group and concordance higher at 3 days or above. In subgroups of normal versus abnormal levels of PCT, it was observed that the levels of CRP were on average higher at less than 2 days compared to patients older than 3 days . Meisner et al, [17] reporting on patients with mechanical trauma noted that PCT and CRP had different time courses for induction. CRP induction had no significant relation to trauma severity and to development of the various stages of sepsis and that the concentrations were elevated for several days after trauma. They also noted that plasma PCT levels declined more rapidly in patients without complications. This perhaps explains higher levels of CRP in patients less than 2 days old by comparison with patients greater than 3 days old.

It has been reported that trends in serum PCT levels over time may be more informative than the first PCT level when this marker is used to make decisions regarding antibiotic administration. [18] PCT has a shorter half-life span than CRP, hence reflects better outcome and response to therapy. [7] Studies have observed an association of systemic infections with significant elevation of PCT. [4] Kofteridis et al, [13] reported that in adults PCT was not a proven marker of bacterial upper respiratory tract infections; while CRP could be used as a marker of these types of infections. Studies have also reported that infections with gram positive bacteria may produce delayed response and perhaps previous antibiotic therapy.[4] The accuracy of PCT in comparison with other markers such as CRP and WCC in newly-born babies indicates early-onset of sepsis i.e. a severe bacterial infection ensuring in the first 3 days of life, still remains unclear in part due to the increase in the level of physiological PCT in the first 2 days [6].

The high CRP and PCT levels in older babies could be due to the fact that most patients over 3 years that were tested for CRP and PCT perhaps had inflammation, but less so in neonates. Given this possibility, correlation would be very unlikely in neonates. Correlation analysis performed on neonates with and without sepsis shows that in neonates, the three indices show no correlation ($r < 0.01$) with one another. Over one month of age, there are observable weak to moderate correlations, which are not consistently decreasing or increasing with age. As mentioned, in neonates, the tests could have been for screening rather than diagnosis or follow up, there may be no substantial cellular injury or inflammation to induce increase of PCT and CRP levels. Hence CRP, but not PCT, that is quite ubiquitous tends to show good correlation with WCC in the absence of sepsis. In non-neonates, the increase of PCT and CRP levels vary during inflammation. PCT increases only moderately in most cases and peaks at 1 to 2 days after cellular injury; the concentrations rapidly declining thereafter and CRP ubiquitously increases and shows no positive correlation. [17] Despite the inconsistencies and/or level of correlations, PCT is more correlated to WCC compared to CRP. [19] In sepsis whereby cellular injury and inflammation would result in increase in CRP and PCT levels, our results show moderate correlation ($r = 0.69$) between the two biomarkers. WCC is moderately correlated with CRP in sepsis, but not with PCT. It is noteworthy that this correlation between WCC and CRP is also observed in clinically healthy neonates who have normal leukocyte counts and no sepsis. Our reference ranges for WCC in paediatrics and children fluctuates with highest levels at birth, decreasing in the first week, slightly increasing in the first year followed by gradually decrease till puberty. Therefore, it is unlikely that biomarkers induced by inflammation such CRP or PCT would have consistent or significant correlations with WCC. When compared with results of blood culture, CRP was more sensitive than PCT for the diagnosis of sepsis, although the very low specificities of both tests make them less helpful in ruling in sepsis. The researchers are mindful that some patients acquire medications from their GPs or across the counter and also seek alternative therapies. However, we did not ascertain the extent of impact on patients put on antimicrobial treatment before blood culture is carried out; which could have affected the study. The sample size was small and confined to 3 hospitals. Other factors such as gestational age at birth and birth weight were not measured and could have had a bearing on the study. This study was not a randomised study and as such the findings are explorative. Future, better designed studies may be able to investigate the diagnostic accuracy of both CRP and PCT in neonates fully.

WCC and CRP are more used because they are widely available, easy to carry out and cost effective, compared to PCT. There is still need to investigate better biological markers for investigating sepsis in neonates. Again there are problems of non-specific nature of symptoms of infection as well as influences of baby weight and variation of the reference ranges.

Conclusion

The data shows that both CRP and PCT in neonates may not be beneficial for the diagnosis of sepsis if used independently. The changes in the levels of PCT from birth up to 2 days makes it difficult to utilise PCT only for the diagnosis. Apart from blood culture results, PCT and CRP can be important diagnostic tools for sepsis.

What is already known on this topic?

- PCT is good marker for sepsis
- CRP is non specific

What this study adds?

- This was the first study to be done in African paediatric intensive care units
- First study to demonstrate the relationship between bacterial species to PCT levels.

Competing interests

The authors declare no competing interest.

Authors' contributions

DONALD MOSHEN TANYANYIWA, conceptualised the study, writing up, revision and approval of final version
JEANNETTE WADULA, Microbiology expertise on samples cultured in her department, revised first and second drafts.
JACQUI BROWN, ICU consultant, recruitment of patients from her unit and revision of first draft.
STEMBISO VELAPHI, NICU consultant and HOD of Paediatrics, recruitment of patients from his unit and revision of first draft.
MANI KHOOSAL, Microbiology technical work and revision of final draft.

Acknowledgements

We are grateful to the following people for their contribution:
Martin Kroon of Mowbray maternity hospital in Cape Town for the careful observation that despite the presence of clinical features consistent with sepsis in the neonates in post-natal ward, most of his CRP request came back normal. His comments resulted in the introduction of PCT test at Red Cross Hospital by the first author. Philip Taderera Bwititi and UE Nwose of School of Biomedical Sciences, Faculty of Science, and Charles Sturt University, New South Wales Australia assisted with the initial statistics. Mani Kossal for the

retrieval and computation of the Microbiology reports and collaborated with Buyisiwe Nkosi in Chemical Pathology.

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