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Original Research Paper

Pathology

ASSOCIATION BETWEEN VERY HIGH PROCALCITONIN AND UNDERLYING BACTERIA.

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ABSTRACT

Objective: The aim of this study is to assess whether there is an association between very high (>100µg/L) procalcitonin (PCT) and type of bacteria in patients being investigated for sepsis.

Methods: A retrospective study in which PCT and blood culture results retrieved from Chemical Pathology and Microbiology respectively were analysed. The study included 160 patients with PCT results <40 PCT ng/L and 120 PCT results >100ng/L selected using a simple purposive sampling technique.

Results: Based on the predominantly cultured microorganism, three different groups were established, Group I comprised 74 patients with Gram-negative culture, Group II comprised 42 patients with Gram-positive culture and Group III comprised 44 patients with negative or no growth after 5 days of culture.

Conclusion: Serum PCT levels were significantly higher in patients with Gram-negative sepsis than in patients with Gram-positive. We have therefore provided evidence that very high PCT levels could tentatively be used as a biomarker to distinguish Gram-negative sepsis from Gram-positive.

KEYWORDS : Gram negative, Gram positive, Procalcitonin, Blood culture

Introduction

Bloodstream infections (BSIs) often result in sepsis. Sepsis is the pathophysiological overwhelming and life-threatening response to infection resulting in morbidity and mortality. Procalcitonin (PCT) has been used to detect the presence of sepsis, while blood culture identifies the microorganisms circulating in the bloodstream [1][2]. Technological advances have resulted in the ability to more rapidly identify aetiological agents of blood system infection, but conventional blood culture methods remain the gold standard for the identification of the organism [3].

Bacteria is broadly be classified into Gram positive and Gram negative (Figure 1 and Figure 2 below) based on their ability to retain primary stain (crystal violet or methylene blue) after treatment with a decolouriser [4]. The difference in the cell wall biochemistry of the microorganism provides the ability for the differentiation. Gram-positive bacteria have a lipid membrane containing a thick layer (20–80 nm) of peptidoglycan compared to the gram-negative bacteria with thin layer (2–3 nm) of peptidoglycan [5]. The cell wall takes up the crystal violet, which is fixed by the addition of iodine. Subsequent addition of a decolorizer, a mixture of ethanol and acetone solvent, dissolves the lipid layer from the gram-negative cells resulting in the release of the primary stain from the cells into the surrounding solvent. In contrast, the solvent dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration resulting in retaining the primary stain [6].

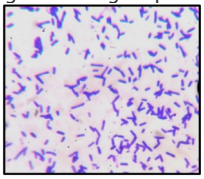

Figure 1. Gram positive bacteria

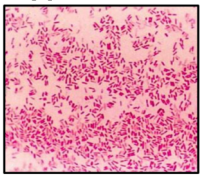

Figure 2. Gram negative bacteria

Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, produced by the C cells of the thyroid gland. Procalcitonin (PCT) is a 116 amino acid prohormone with a molecular weight of approximately 12.7kD. Identification of elevated levels of PCT in patients post thyroidectomy suggested that PCT was associated with sepsis and was produced elsewhere. Peripheral blood mononuclear cells (PBMCs) have been shown to produce PCT [7]. This production is strongly associated with inflammatory cytokines (TNF-α, IL-6, IL-2) and bacterial endotoxins and exotoxins [8]. The first determination of PCT was in 1993 to differentiate bacterial from viral meningitis [9].

The difference between the Gram-negative and Gram-positive bacteria membrane biochemistry is responsible for the mechanism underlying the PCT production. The main membrane component in Gram-negative bacteria is lipopolysaccharide (LPS) which is the major component of endotoxin. In Gram-positive bacteria, the main component is peptidoglycan (PGN) [10]. LPS and PGN are sensed as different receptors, which trigger different responses after binding to their respective ligands. Binding of the Gram-negative LPS activates and induces the production of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Mononuclear blood cells produced are known to mediate the expression of serum PCT concentrations in response to Gram-negative bacterial infections [11].

2. Method

2.1 Study population

Study design

A retrospective study carried out in the departments of Chemical Pathology and Medical Microbiology at Chris Hani Baragwanath Academic Hospital between 2010 and 2011. The study included 160 patient PCT results made up of 40 PCT results <0.5ng/L and 120 PCT results ≥100ng/L selected using a simple purposive sampling technique. These laboratories serve the largest tertiary academic

hospital in the southern hemisphere and more than 30 surrounding clinics. They are accredited by the South African National Accreditation System (SANAS) and participates in the National Health Laboratory Services EQA program.

Inclusion criteria of the study

(1) PCT results <5ng/L
(2) PCT results>100ng/L
(3) Patients with blood culture results taken <48hrs of the PCT test.

2.2 Analytical procedures

PCT Analysis

Blood samples collected into Becton, Dickinson (BD) serum separator tubes for the determination of PCT. Samples were centrifuged at least 30 minutes after collection and analysed immediately. Measurements of PCT was on a Roche Modular diagnostic platform according to the specification of the manufacturers: Roche Diagnostics (Risch-Rotkreuz, Switzerland).

Blood Culture

Blood cultures were processed using the automated BacT/Alert system (BioMériueux, inc, Durham). Microscopy, culture and susceptibility testing were done on all positive cultures using the Kirby-Bauer method as described in the clinical laboratory standard institute (CLSI) guidelines.

2.4 Data Analysis

Microsoft Excel was used to capture the PCT levels data. Basic descriptive statistics were used to describe the basic features of the data in a study. It provided simple summaries and graphic analysis about the samples. The Clinical cut-offs with the Elecsys BRAHMS PCT assay on the Roche platform are PCT values: < 0.5 ng/mL represent a low risk of severe sepsis and/or septic shock > 2.0 ng/mL represent a high risk of severe sepsis and/or septic shock

3. Results

One hundred and sixty consecutive patients were included using a simple purposive sampling technique. Based on the predominantly cultured microorganism, three different groups were established shown in Figure 3 below. Group I comprised 74 patients with Gram-negative cultures, Group II comprised 42 patients with Gram-positive cultures and Group III comprised of 44 patients with negative or no growth after 5 days of culture. Based on PCT levels, the three groups were catergorised into two, 123 patients with PCT levels >100ng/ml and 37 with PCT levels <0.5ng/ml, shown is Figure. 4 and Figure.5 respectively.

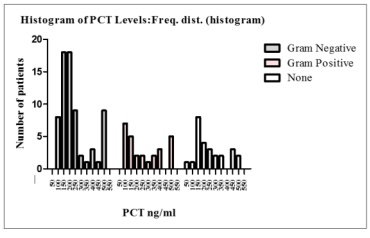

Figure. 3. Three different groups based on cultured organisms.

The result show that for the Gram negative organisms, most patients had PCT levels between 100 and 250ng/ml and 10/74 (14%) had 500ng/ml. Patients with Gram positive organism have PCT levels between 100 and 150ng/ml and similarly 5/42 (12%) had PCT levels of 500ng/ml. 9/44 (20%) of those with negative cultures had PCT levels of 150ng/ml.

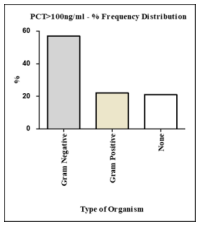

Figure. 4. Percent frequency distribution for >100ng/ml PCT.

The result show that for PCT >100ng/ml, patients with gram negative bacteria, those with Gram negative organisms, constituted almost 60%, Gram positive 36% and negative/no growth 34%. 20 of the 44 patients (45%) with negative/ no growth had TDM requests implying that the measure antibiotic had been administered.

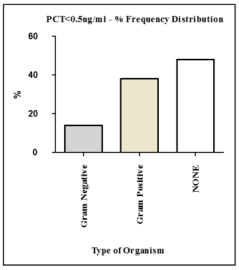

Figure. 5. Percent frequency distribution for >100ng/ml PCT.

The result show that for PCT<0.5ng/ml, patients with negative/no growth constituted 47% followed by Gram positive at 37% and Gram negative at 16%

4. Discussion

We previously reported the combined effects of both CRP and PCT for the diagnosis of sepsis in neonates. This study correlated PCT levels to the two broad types of bacteria. The main findings of this study are that, in patients with suspected sepsis, high PCT >100µg/ml could be used in predicting an infection caused by Gram-negative bacteria. This is useful to clinicians when deciding on appropriate initial antimicrobial therapy. Other studies identified Gram-negative bacteria as a cause bloodstream infection, [12] [13] but did not correlate the microorganisms to PCT levels. The study confirms reports by several studies that Gram-negative bacterial infections had higher PCT than Gram-positive infections and further reported differences within the family of Gram-negative bacteria [14]. Induction by endotoxin can result in PCT increase more than a hundred fold in 2 to 3 hours, reaching a peak level between 6 and 12 hours. PCT concentrations remain high for up to 48 hours, falling to their baseline values within the following 2 days [15]. The half-life is about 20 to 24 hours. Data retrieved from the therapeutic drug monitoring (TDM) requests found that 20 of the 44 patients (45%) with negative/no growth after 5 days were on antibiotic. 16 of the 20 (80%) patients with TDM requests had requests for vancomycin, 3 (15%) had requests for Amikacin and 1(5%) had a request for gentamycin. Therefore, the study confirms that the use of antibiotics before obtaining blood cultures results in loss of pathogen detection [16] [17]. Based on the PCT half-life, the blood cultures with negative growth but high PCT levels must have been done within the half-life period. Nearly 50% of patients with <0.5ng/ml PCT had negative/no growth results as expected while those with Gram positive cultures constituted 37% and Gram negative was the least in this category. The Gram positive culture were predominantly positive cocci, coagulase negative staphylococci, a finding associated with blood culture contamination [18].

5. Conclusion

Serum PCT levels were significantly higher in patients with Gram-negative sepsis than in patients with Gram-positive sepsis. We have provided evidence that PCT can be used as a biomarker to distinguish Gram-negative sepsis from Gram-positive. Therefore, in the absence of blood cultures, antimicrobial agents known to be specific for these microorganisms can be selected. The reports of negative cultures in the presence of significant PCT levels is due to collection of blood samples within 24 hours of antimicrobial commencement.

Recommendations

Blood culture remains the gold standard for microorganism positive identification followed by drug sensitivity testing. To reduce

inappropriate use of antibiotics, we recommend venesection for blood culture immediately after a clinical diagnosis of sepsis to facilitate early commencement of antibiotics and strongly support the current recommendation to obtain blood cultures before antibiotic administration in patients with sepsis.

Strength and Limitations

The study looked at 160 PCT and blood culture results. Our study has several limitations, including a relatively small sample size; single center study and did not stratify the bacteria. Retrospective studies are generally less expensive than prospective studies but their limitations include selection and information bias, and limitations in the completeness and availability of data. Even though the number is small, the study adds value to the limited number of studies from Africa relating PCT to bacteria.

Competing interests: None

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Ethical approval-: CHBAH Medical Advisory Committee, Department of Health and University of the Witwatersrand Ethics Committee.

REFERENCES

1. Lee, A., Mirrett, S., Reller, L.B. and Weinstein, M.P. 2007. Detection of bloodstream infections in adults: how many blood cultures are needed? Journal of Clinical Microbiology, 45:p3546–3548
2. Weinstein, M.P. 1994. Clinical importance of blood cultures. Clinical Laboratory Medicine, 14: p9-16.
3. Lagace-Wiens, P.R., Adam, H.J. and Karlowsky, J.A. 2012. Identification of blood culture isolates directly from positive blood cultures by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and a commercial extraction system: Analysis of performance, cost, and turnaround time. Journal of Clinical Microbiology, 12 50:p3324–3328.
4. Gram, C. 1884. The differential staining of Schizomycetes in tissue sections and in dried preparations. Fortschitte der Medicin, 2: p185-189.
5. Gupta, R. S. 1998. "What are archaebacteria: life's third domain or monoderm prokaryotes related to Gram-positive bacteria? A new proposal for the classification of prokaryotic organisms". Molecular Microbiology, 29 3: p695–707.
6. Claus, D., 1992. A standardized Gram staining procedure. World journal of Microbiology and Biotechnology, 8 4: p451-452.
7. Oberhoffer, M., Stonans, I., Russwurm, S., Stonane, E., Vogelsang, H.J.U., Jager, L. and Reinhart K. 1999. Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis-related cytokines in vitro. Journal of Laboratory and Clinical Medicine, 134: p49-55.
8. Kristoffersen, K.B., Sogaard, O.S., Wejse, C. and Black, F.T. 2009. Antibiotic treatment interruption of suspected lower respiratory tract infections based on a single procalcitonin measurement at hospital admission-a randomized trial. Clinical Microbiology and Infection, 15: p481-487.
9. Assicot, M., Gendrel, D., Carsin, H., Raymond, J., Guilbaud, J and Bohuon, C. 1993. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet, 27 341(8844): p515-8.
10. Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H. and Ogawa, T. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity, 11:p443–51.
11. Beran, O., Potmešil, R. and Holub, M. 2011. Differences in Toll-like receptor expression and cytokine production after stimulation with heat-killed gram-positive and gram-negative bacteria. Folia Microbiologica (Praha), 56:p138–42.
12. Lochan, H., Pillay, V., Bamford, C., Nuttall, J. and Eley, B. 2017. Bloodstream infections at a tertiary level paediatric hospital in South Africa. BioMed Central Infectious Diseases, 17 (1) 750:p2-9.
13. Berkley, J.A., Lowe, B.S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., Ngetsa, C., Slack, M.P., Njenga, S., Hart, C, A., Maitland, K., English, M., Marsh, K. and Scott. J.A. 2005. Bacteremia among children admitted to a rural hospital in Kenya. New England Journal of Medicine, 352(1):p39-47.
14. McDonald LC, Fune J, Gaido LB. 1996. Clinical importance of increased sensitivity of bact/alert fan aerobic and anaerobic blood culture bottles. Journal of Clinical Microbiology, 34: p2180–2184.
15. Meisner, M. 2014. Update on Procalcitonin Measurements. Ann Lab Med. 34 (4): p263–273.
16. Scheer, C.S., Fuchs, C., Grundling, M., Vollmer, M., Bast, J., Bohnert, J.A., Zimmermann, K., Hahnenkamp, K., Rehberg, S. and Kuhn, S.O. 2018. Impact of antibiotic administration on blood culture positivity at the beginning of sepsis: a prospective clinical cohort study. Clinical Microbiology Infection. pii: S1198-743X (18)30449-X.
17. Shallcross, L. J., Freemantle, N., Nisar, S. and Ray, D. 2016. A cross-sectional study of blood cultures and antibiotic use in patients admitted from the Emergency Department: missed opportunities for antimicrobial stewardship. BioMed Central Infectious diseases, 16, p166.
18. Hall, K.K. and Lyman J, A. 2006. Updated Review of blood culture contamination. Clinical Microbiology Reviews, 19 4: p788-802