Evaluation of the Utilisation of Semi-Quantitative Procalcitonin versus C-Reactive Protein for the diagnosis of infection in a Hospital Paediatric Population, with Particular Reference to Utilisation in Suspected Bacterial Meningitis

By

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As the candidate's supervisor I have approved this thesis for submission.

Signed:  Name: Verena Gounden Date: 6 June 2019

Declaration

I.....Unathi Ngxamngxa.....declare that

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Dedication

To my parents, who have been so supportive of my academic work.

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- My Supervisor, Dr Verena Gounden for her guidance and assistance in compiling the thesis
- King Edward VIII Hospital for granting permission to perform the study.
- NHLS Central Data Warehouse for providing data used for the study

Overview of the thesis

Procalcitonin (PCT) and C-Reactive protein are important markers of infection. PCT has been shown to be more specific for bacterial infection than CRP. However fully quantitative PCT is often only available at academic hospital laboratories particularly in the KwaZulu Natal region. Semi-quantitative PCT provides an alternative option to provision of this test that does not require further specialized equipment or training.

This project aims to review the utility of semiquantitative PCT versus CRP in comparison to findings on culture in a hospitalized paediatric population, in particular those with suspected bacterial meningitis. Although PCT and CRP have been studied extensively with regards to their diagnostic and prognostic value, few of these studies have been performed in Africa and no known studies have been done looking at the utilisation of semi quantitative PCT in this setting.

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Part 1: The Review of Literature

Procalcitonin

Procalcitonin (PCT) was introduced as a biomarker of sepsis in the early 1990s. [1] PCT is a 116 amino acid polypeptide precursor of calcitonin, which is synthesized in the thyroid C cells. Calcitonin inhibits bone resorption during hypercalcaemic states. [2,3] The cleavage of the three-part precursor molecule prePCT to the mature calcitonin occurs in the C cells of the thyroid, and in other neuroendocrine cells. [4] Other tissue types lack the ability to further cleave immature PCT to the mature calcitonin. [4] Procalcitonin constitutes of three sections; the amino terminus, immature calcitonin and the carboxyl terminus CCP-1. Pre-PCT is cleaved into PCT which is further broken down into mature calcitonin. The production of PCT is under the control of calcitonin 1 gene (CALC-1) which is located on chromosome 11.[4]

PCT is an immunologically active protein which has tightly regulated levels and hence differs from other inflammatory molecules such as cytokines, acute phase proteins and leucocyte surface markers. It is a good marker of infections secondary to bacterial infections and is produced by many tissues such as the liver, kidney and the pancreas in response to inflammation. [5,6]

It is one of the acute phase proteins which rises rapidly in response to inflammation, followed by CRP, serum Amyloid A and α_1 antichymotrypsin. These proteins also show different rates of decrease in the same order. [6] Acute phase reactions to bacterial infections, are more pronounced than those seen in viral infections. [6] It has been noted that interferon-γ which is produced in viral infections, suppresses the production of PCT, enabling the biomarker to differentiate between the two types of infection, and to be more specific for bacterial infections. [4]

Local or systemic infection results in PCT production, in response to cytokines such as interleukin-6, interleukin-1β and tumour necrosis factor α, which are produced by monocytes. [4] The biological effects of PCT include chemokine effects, stimulating release of proinflammatory

cytokines and modulating vascular contraction. [5] It has been demonstrated that serum PCT levels increase during the course of bacterial, parasitic, or fungal infections, but remain normal or slightly increased in viral infections and inflammatory reactions that are not infectious. [7] PCT differs from other markers of sepsis such as CRP, lactate and cytokines in that it is more specific for sepsis, and can also indicate the severity of sepsis. [3]

Elevated PCT levels may also be due to non-infectious causes such as chronic kidney disease, in paraneoplastic syndromes due to medullary thyroid cancer and use of therapeutic drugs such as alemtuzumab and Il-2. These drugs potentiate the release of cytokines. [4]

Non-thyroidal PCT production was noted in a study by *Morgenthaler et al*, where lipopolysaccharide(LPS) was injected in baboons with absent thyroid glands. A noted response of increased PCT levels was noted within 3-5 hours post injection. PCT concentrations of greater than 0.2ng/g of wet tissue, were noted in organs such as liver, kidney, the aorta and adrenals. The findings of this study illustrated the origin of extra-thyroidal PCT in sepsis.[8]

A study by Dipalo *et al* which compared different PCT assays to the BRAMHS PCT Kryptor, showed optimum agreement at three diagnostic thresholds for bacterial infections. [9] The agreement noted translates into the use of the same PCT cut-offs. [4] In the healthy population, the PCT concentration is usually <0.05ug/L, while the concentration is usually higher in the newborn period.[4] An increase in PCT is seen in infections, and the increase usually correlates with the severity of the infection. [4] Table 1 below describes the commonly used diagnostic thresholds for PCT.

Table 1 Interpretation of PCT values [4]

PCT value (ug/L)	Interpretation
<0.05	No infection
0.05-<0.5	Systemic infection is unlikely although localised infection is possible
0.5-<2	Systemic infection is possible but other conditions (for example major trauma, recent surgery) may also induce these values
2-<10	Systemic infection is likely
>10	High likelihood of severe bacterial sepsis/septic shock

PCT may be used in various clinical settings such as in primary care, casualty and ICU. [4] It is used to guide antibiotic treatment in conditions such as acute respiratory tract infections, pneumonia and sepsis. [4]

Studies which looked at PCT guided antibiotic use, showed outcomes of up to 42% reduction in antibiotic use as well as a decrease in hospital stay from 7.4 to 5.9 days. [4] This equates to a decrease in health related costs.

Current methods for PCT measurement are immunoassay based. The first commercially available assay was introduced by BRAHMS and this assay has since been adapted by other diagnostic companies for the various platforms. Methods used to measure PCT, need to have high sensitivity and have detection limits as low as 0.02 ng/ml. [5] The semiquantitative or point of care method for PCT (BRAHMS PCT-Q) is based on an immunochromatographic technique and is available as a test strip. This is a one-step immunochromatographic assay (sandwich principle) using immunogold labelling. Immunochromatography represents a combination of chromatography and immunoassay. Briefly, described as an antigen-antibody reaction which occurs on a membrane. The antigen is the analyte of interest to be detected. The antibody against the antigen of interest is impregnated in a membrane usually made of nitrocellulose along with some dyes (immunogold) which produces respective coloured lines according to the presence or absence of target analyte.[10] Results are available within minutes, and it does not require any specialized expertise or equipment to perform the analysis.

Semiquantitative PCT (SPCT) methods have been shown to have 93.94% sensitivity and 87.23% specificity for severe sepsis. [11] According to a study by Oh *et a*l, these semiquantitative methods are fast and effective, and are useful to initiate and guide antibiotic treatment. [12]

Boo *et al* reviewed the usefulness of SPCT for the early diagnosis of neonatal sepsis and found that a negative SPCT could be reliably used as a rule out for sepsis.[11] In another study looking at adult patients with community acquired pneumonia SPCT at admission was found to be an excellent test to predict the outcome of pneumonia. The authors reported a receiver operating characteristic for mortality prediction area under curve of 0.92. [13]. Another study amongst Japanese adult patients determined that the SPCT was as useful for distinguishing bacterial infection from other inflammatory diseases in common clinical practice, as the quantitative PCT.

[14] The semi quantitative PCT method, may aid in diagnosis of a bacterial infection, yet has limited use in monitoring PCT changes over time to review response to treatment. [12]

Fully quantitative PCT analysis is usually only available in academic centers in the South African public health system. Thus the turn-around time for tests referred from other hospitals is often much greater than the 1-3 hours required for the result, in order to effectively manage the patient. However, procalcitonin tests costs are significantly higher than those of CRP. Semi quantitative methods are cheaper, more readily available and do not require expensive instrumentation.

C-reactive protein vs PCT

C-reactive protein (CRP) was discovered in 1930 by Tillet and Francis, who noted that the protein formed a complex with pneumococcal C-Polysaccharide during pneumonia infections. [15] CRP is a sensitive acute-phase reactant for inflammation which is made in the liver, in response to interleukin 6. [15,16] It activates the classical complement pathway. [15]

CRP concentrations increase in response to infection, trauma, surgery and chronic inflammatory conditions. Mild inflammatory conditions and viral infections cause lower CRP production than bacterial infections, with the highest concentrations being found in severe bacterial infections. [15,16]

CRP methods include immunoturbidimetric and fluorescence labeled ELISA methods, which are commonly used. These automated methods offer high sensitivity and specificity. [15]

Traditional inflammatory markers such erythrocyte sedimentation rate (ESR), white cell count and CRP are noted for their poor sensitivity and specificity in bacteremia.[3] Furthermore the gold standard diagnostic test which is microbiology culture has limitations of being time consuming, together with false negative or false positive results. [3]

PCT has shown better sensitivity (88%) and specificity (81%) compared to CRP with a sensitivity of 75% and specificity of 67% in the diagnosis of bacterial infection. [16]
PCT also has an added advantage of being elevated within 2-4 hours, while CRP rises within 12-

24 hours post inflammation. PCT with a half-life of 24 hours, returns to normal levels far earlier than CRP, which may remain high for up to 7 days post recovery. Nargis *et al* demonstrated that the use of PCT reduces the number of days for which antibiotics are taken, and results in a reduced hospital admission. [3]

A study by Hoeber *et al* noted that changes in PCT and CRP were better predictors of the evolution of nosocomial infections, when compared to changes in the white cell count. [17] The authors noted CRP as useful in predicting localized bacterial infections, while PCT has been useful in the prediction of bloodborne bacterial infections, along with their clinical sequelae.[17]

The indiscriminate increase in CRP due to viral or bacterial infections, makes PCT a superior biomarker in order to differentiate viral from bacterial meningitis.[18] Another advantage of measuring PCT instead of CRP, is that use of anti-inflammatory drugs, steroids and inflammatory conditions such as inflammatory bowel disease, cannot affect its concentration. The study by Vikse *et al* mentioned that the limitations of PCT in the diagnosis of bacterial meningitis in adults, included its decreased concentration when antibiotics are used, and an increase in other bacterial infections such as pneumonia and sepsis. [18] Nargis *et al* noted PCT to be a better marker of accuracy than CRP in identifying and in assessing the severity of sepsis. The authors noted that PCT testing can lead to earlier disease diagnosis and along with its use for monitoring, can thus reduce the unnecessary use of antibiotics. [3] A study by Alkholi *et al* showed that PCT levels were significantly higher in patients with bacterial meningitis (mean, 24.8 ng/ml) compared to patients with viral meningitis (mean, 0.3 ng/ml) (P<0.001). The study also demonstrated that PCT levels in bacterial meningitis group decreased sooner in those children after treatment initiation.[7]

Like the studies described above, many of the studies reviewing performance of PCT and CRP have used fully quantitative PCT analysis. One study by Boo *et al* examining utility in neonatal sepsis found that whilst negative SPCT was a good "rule-out" test, and a raised CRP performed better as a "rule-in" test for the possibility of sepsis. [10] In another study the areas under the resulting curves for SPCT (0.764) were significantly larger than those seen for C-reactive protein (0.650, p= 0.02) and white blood cells (0.618, p= 0.006) for the detection of bacterial infection. In an ICU setting SPCT was shown to be more specific and sensitive than CRP for the diagnosis of sepsis in critically ill patients. [19]

The diagnosis of bacterial infections is important, as they are a significant cause of morbidity and mortality. [16] The worldwide annual incidence is 1.2 million cases/year, with a 135 000 deaths.[18] In the South African paediatric population the yearly incidence of bacterial meningitis is 40 per 100 000 in the <1 year age group, and 7 per 100 000 in the 1-4 year age group [20] In children it is often difficult to determine whether the aetiology of meningitis is viral or bacterial. Early diagnosis of bacterial meningitis is critical in order to initiate appropriate therapy, and to prevent the unnecessary and widespread use of antibiotics in cases of viral meningitis [4,21]

Distinguishing bacterial and viral meningitis early could also help to reduce hospital admissions. Use of clinical criteria, Gram staining, and bacterial antigen testing of CSF as well as the inflammatory markers in the blood (CRP level, white blood cell count [WBC], and neutrophil count) or CSF (protein level, glucose level, WBC count, and neutrophil count) used alone do not offer 100% sensitivity with high specificity for distinguishing bacterial from viral meningitis. Results of CSF culture may take a number of days and detection of viral agents using PCR is not readily available in most centers. [7]

The diagnostic challenge posed by similar clinical features of bacterial and viral infections makes the role of biomarkers such as PCT critical in identifying bacterial infections. [7] In young children the clinical features of meningitis may differ according to age, and are often not the classical signs of meningitis found in older children and adults. This poses a diagnostic challenge, and makes the role of tests such as CSF chemistry and bacterial culture, blood culture and serum PCT or CRP and peripheral white cell count imperative in making the diagnosis. [20] The bacterial meningitis score (BMS) is useful in differentiating bacterial from viral meningitis. The score is negative when the following are absent; CSF gram stain, CSF neutrophil count ≥ 1000 cells/mm^3. CSF protein ≥0.8 g/l, peripheral blood absolute neutrophil count ≥ 10 000 cell/mm^3 and history of seizures peri-onset of presentation. [3] According to Boyles et al, when the BMS is negative, the negative predictive value is 99.9%. The limitation for the score is that it is not validated to use in certain patient groups; such as infants of less than 2months of age and in immunocompromised patients amongst others. [18]

CRP testing is readily available in most labs in South Africa, whilst PCT is limited to the large urban centers and academic laboratories. The use of semi-quantitative, point of care PCT analysis using immunochromatography technology allows for this testing to be accessible to all patients in less well-resourced health care facilities. There is however the limitation of prior centrifugation of the patient sample, which makes the test impractical in rural point of care settings.

There are very few studies performed in an African population reviewing the utility of PCT and the authors could not find any studies in this population that examined the utility of semi-quantitative PCT measurements.[22, 23. 24]

The aims of this study were to a) describe the utilisation of semiquantitative serum PCT levels in the diagnosis of bacterial infections in a South African paediatric population and b) to compare its utility in a subset of patients to aid in diagnosis of bacterial meningitis

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Part 2: A submission ready manuscript.

Title: Evaluation of the Utilization of Semi-Quantitative Procalcitonin versus C-Rreative Protein for the Diagnosis of Infection in a Hospital Paediatric Population, with Particular Reference to Utilisation in Suspected Bacterial Meningitis

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Abstract:

Background:

The high mortality and morbidity due to bacterial infections such as meningitis, alongside the long turnaround times for gold standard microscopy and culture testing, warrants alternative laboratory tests such as C-reactive protein (CRP) and procalcitonin(PCT), which demonstrate adequate test sensitivity and specificity. In addition, there is a need for more studies a in Africa, which will show the diagnostic value of CRP and PCT in bacterial infections. The aims of this study were a) to describe the utilisation of semiquantitative serum PCT in aiding with the diagnosis of bacterial infections in a South African paediatric population and b) to compare the utility of PCT in a subset of patients with suspected bacterial meningitis.

Methods

A retrospective observational study with charts review was done. It included data for all paediatric patients admitted to the King Edward Hospital (Durban, South Africa) for which semi quantitative PCT testing was performed for the period April 2013- April 2016. Descriptive statistical methods were employed for the analysis of data.

Results

The semi-quantitative PCT results correlated well with the serum CRP levels and showed significant correlation with extent of rise in CRP. A statistically significant ($p=0.035$) difference of median CRP levels across different categories of PCT levels, with higher values associated with PCT categories associated with greater degrees of infection. Kappa statistic analysis performed to determine agreement between positive and negative culture results with positive and negative semi quantitative PCT results, showed poor observed agreement of 53.13% (acceptable >75%).

Conclusions

Findings of the current study do not demonstrate added benefit of use of semi-quantitative PCT over traditional CRP. Further studies will need to be done to examine utility of the

semiquantitative PCT test for early diagnosis specific bacterial infections including meningitis and pneumonia in this specific population.

Background:

Procalcitonin (PCT) was introduced as a biomarker for sepsis as early as 1993. [1] PCT is a 116 amino acid polypeptide precursor of calcitonin, which is synthesized in the thyroid C cells. [2,3]

PCT is an immunologically active protein which has tightly regulated levels and hence differs from other inflammatory molecules such as cytokines, acute phase proteins and leucocyte surface markers. [4]

Local or systemic infection results in PCT production in response to monocytes. The biological effects of PCT include chemokine effects, stimulating release of pro-inflammatory cytokines and modulating vascular contraction. [4] It has been demonstrated that serum PCT levels increase during the course of bacterial, parasitic, or fungal infections, but remain normal or slightly increased in viral infections and inflammatory reactions that are not infectious. [5] PCT differs from other markers of sepsis such as CRP, lactate and cytokines in that it is more specific for sepsis and can also indicate the severity of sepsis. [1]

PCT measurements are performed on immunoassay based methods. They are quantitative or semi-quantitative, and are measured in serum or plasma. Methods used to measure PCT need to have high sensitivity and have detection limits as low as 0.02 ng/ml. [4] Semi-quantitative PCT methods have been shown to have 93.94% sensitivity and 87.23% specificity for severe sepsis. [6] According to a study by Oh J *et al*, these semi-quantitative methods are fast and effective, and are useful to guide antibiotic treatment. [6]. Fully quantitative PCT analysis performed on an immunoassay analyser is usually only found in the larger academic and tertiary regional hospital laboratories. However, quantitative procalcitonin tests costs are significantly higher than those of CRP. Semi-quantitative methods are cheaper, more readily available and do not require expensive instrumentation.[7]

C- reactive protein (CRP) is a sensitive acute-phase reactant for inflammation which is made in the liver. It activates the classical complement pathway. [7] CRP concentrations increase in response to infection, trauma, surgery and chronic inflammatory conditions. Mild inflammatory

conditions and viral infections cause lower CRP production than bacterial infections, with the highest concentrations being found in severe bacterial infections. [7] CRP methods include immunochemical techniques such as immuno -turbidimetric and immuno-nepholometry both of which are commonly used. Traditional inflammatory markers such erythrocyte sedimentation rate (ESR), white cell count and CRP are noted for their poor sensitivity and specificity in bacteraemia.[3] Furthermore the gold standard diagnostic test which is microbiology culture has limitations of being time consuming, together with false negative or false positive results. [3]

PCT has shown better sensitivity (88%) and specificity (81%) compared to CRP with a sensitivity of 75% and specificity of 67% in the diagnosis of bacterial infection. [8]
PCT also has an added advantage of being elevated within 2-4 hours, while CRP rises within 12-24 hours post inflammation. PCT with a half-life of 24 hours, returns to normal levels far earlier than CRP, which may remain high for up to 7 days post recovery. Nargis *et al* demonstrated that the use of PCT reduces the number of days for which antibiotics are taken, and results in a reduced hospital admission. [3] A study by Hoeber *et al* noted that changes in PCT and CRP were better predictors of the evolution of nosocomial infections, when compared to changes in the white cell count. [9] The authors noted CRP as useful in predicting localized bacterial infections, while PCT has been useful in the prediction of blood-borne bacterial infections, along with their clinical sequelae.[9]

The indiscriminate increase in CRP due to viral or bacterial infections, makes PCT a superior biomarker in order to differentiate viral from bacterial meningitis.[10] Another advantage of measuring PCT instead of CRP, is that use of anti-inflammatory drugs, steroids and inflammatory conditions such as inflammatory bowel disease, cannot affect its concentration. Vikse *et al* stated that one of the limitations of PCT in the diagnosis of bacterial meningitis in adults, included its decreased concentration when antibiotics are used, and an increase in other bacterial infections such as pneumonia and sepsis. [10] Nargis *et al* noted PCT to be a better marker of accuracy than CRP in identifying and in assessing the severity of sepsis. The authors noted that PCT testing can lead to earlier disease diagnosis and along with its use for monitoring, can thus reduce the unnecessary use of antibiotics. [3]

One study by Boo *et al* examining utility in neonatal sepsis found that whilst negative SPCT was a good “rule-out” test, a raised CRP performed better as a “rule-in” test for the possibility of

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CRP testing is readily available in most labs in South Africa, whilst PCT is limited. The use of semi-quantitative point of care PCT analysis using immuno-chromatography technology allows for this testing to be accessible to all patients in less well-resourced health care facilities.

The diagnosis of bacterial infections is important, as it confers a significant cause of morbidity and mortality. [8] The worldwide annual incidence is 1.2 million cases/year, with a 135 000 deaths.[10] In the South African paediatric population the yearly incidence of bacterial meningitis is 40 per 100 000 in the <1 year age group, and 7 per 100 000 in the 1-4 year age group[13] In children it is often difficult to determine if the aetiology of meningitis is viral or bacterial. Early diagnosis of bacterial meningitis is critical in order to initiate appropriate therapy and to prevent the unnecessary and widespread use of antibiotics in cases of viral meningitis [8,14]

Distinguishing between bacterial and viral meningitis early could also help to reduce hospital admissions. A study by Alkholi *et al* showed that PCT levels were significantly higher in patients with bacterial meningitis (mean, 24.8 ng/ml) compared to patients with viral meningitis (mean, 0.3 ng/ml) (P<0.001). The study also demonstrated that PCT levels in bacterial meningitis group decreased sooner in those children after treatment initiation. Use of clinical criteria, Gram staining, and bacterial antigen testing of CSF as well as the inflammatory markers in the blood (CRP level, white blood cell count [WBC], and neutrophil count) or CSF (protein level, glucose level, WBC count, and neutrophil count) used alone do not offer 100% sensitivity with high specificity for distinguishing bacterial and viral meningitis. Results of CSF culture may take a number of days and detection of viral agents using PCR is not readily available in most centers. [5]

The diagnostic challenge posed by similar clinical features of bacterial and viral infections makes the role of biomarkers such as PCT critical in identifying bacterial infections. [5] In young children the clinical features of meningitis may differ according to age and are often not the

classical signs of meningitis found in older children and adults. This poses a diagnostic challenge and makes the role of tests such as CSF chemistry and bacterial culture, blood culture and serum PCT or CRP and peripheral white cell count imperative in making the diagnosis. [13] The bacterial meningitis score (BMS) is useful in differentiating bacterial from viral meningitis. The score is negative when the following are absent; CSF gram stain, CSF neutrophil count ≥ 1000 cells/mm^3 , CSF protein ≥0.8 g/l, peripheral blood absolute neutrophil count ≥ 10 000 cell/mm^3 and history of seizures peri-onset of presentation. [1]. According to Boyles et al, when the BMS is negative, the negative predictive value is 99.9%. [13] The limitation for the score is that it is not validated to use in certain patient groups; such as infants < 2months and immunocompromised patients amongst others. [13]

There are few studies that have investigated the diagnostic value of semi-quantitative PCT in detection of bacterial infection in Africa and data available with regards to the clinical utility of semi-quantitative PCT for diagnosis of bacterial meningitis is also limited.

The aims of this study were to a) describe the utilisation of semi-quantitative serum PCT in the diagnosis of bacterial infections in a South African paediatric population and b) to review its utility in a subset of patients to aid in diagnosis of bacterial meningitis

Methods

Semi-quantitative PCT results for all paediatric patients admitted to the King Edward Hospital (Durban, South Africa) performed for the period April 2013- April 2016, were extracted from the National Health Laboratory Service (NHLS) laboratory information system central data warehouse (CDW). Demographic data, clinical history (where provided) as well as results for CRP, white cell count, blood culture, CSF chemistry, microbiology and culture studies were also extracted. Sputum culture was not reviewed as part of the study due to the general difficulty with obtaining samples from paediatric patients and high prevalence of pulmonary tuberculosis endemic in the region. Patient chart review was also performed to obtain additional clinical information regarding clinical presentation and management.

Semi-quantitative PCT analysis was performed at the King Edward Hospital National Health Laboratory Service (NHLS) chemical pathology laboratory. The Brahms PCT-Q assay (Thermo Scientific) was used. This method is based on a one-step immuno-chromatographic assay (sandwich principle) using immuno-gold labelling. The test procedure is carried out on non-hemolyzed blood samples that have been centrifuged. 200 µL of serum is pipetted into the round cavity of the test trip. The tracer binds to any PCT in the sample and a marked antigen-antibody complex is formed. This complex moves by means of capillary action through the test system, and in the process, passes through an area containing the test band. Here, the marked antigen-antibody complex binds to the fixed anti-calcitonin antibodies and forms a sandwich complex. This sandwich complex can be seen as a reddish band. The color intensity of the band is indicative of the level of PCT. Results are categorised into four different grades (Grade 0:< 0.5; Grade1: 0.5 to < 2.0, Grade2: 2.0 to < 10.0, or Grade 3: ≥ 10.0 ng/mL).

CRP was measured using an immuno-turbidimetric based assay on the Beckman Coulter DXC800 analyser.

Suspected meningitis cases were identified based on the documented symptoms and assessment by the attending clinicians as per chart review and laboratory requests for CSF biochemistry and microscopy (with or without CSF culture)

The following variables were retrieved from review of patient charts 1) presence of coexisting illness; 2) clinical data with regards to signs and symptoms present 3) treatment details specifically with regards to initiation of antibiotic treatment during the admission 4) differential diagnosis 5) where possible clinical outcomes

Patients were identified with a unique hospital number on the extracted database. With regards to repeat testing only the initial CRP and PCT values were utilized for analysis in this study.

Inclusion criteria**:**

All paediatric patients between 0 months and 12 years admitted during the period from 1 April 2013 – 1 April 2016 that had both semi quantitative procalcitonin and CRP testing performed within 24 hours of each other.

Exclusion criteria

- **Any patient > 12 years old**
- **Patients with no CRP performed within 24 hours of PCT**
- **Outpatients**

Statistical analysis: Data was analysed using Medcalc statistical software program version 18.11 (Medcalc, Belgium). Differences between groups in categorical variables were tested for significance with the Kruskal Wallis test. Differences in frequencies of findings between groups were analyzed by Fischer's exact test. The chi-square test of association was used to assess any associations between categorical variables. Kappa statistics was used to compare the level of agreement between tests. A p value <0.05 was considered as statistically significant. Ethical approval for the study was obtained from the University of KwaZulu Natal Bioethics and Research Committee (BREC) (approval number BE404/16)

Results

During the period of review, a total of 1281 semi-quantitative PCT tests were performed for the paediatric population ≤ 12 years of age. A total of 49 patients were identified as fulfilling the inclusion criteria following review of extracted data. Results from 49 patients are presented. Demographic data and patient summary characteristics are presented in **Table 1** below.

Table 1 Patient summary characteristics

	Male	**Female**	**Total**
Number	**30**	**19**	**49**
Age (months) (Median range)	0.36 (0.03- 132.07)	1,23 (0.03-118.9)	0.7 (0.03- 132.07)
PCT (ng/mL)**:** Grade 0(<0.5 ng/mL)	13	8	21
Grade 1(>0.5<2 ng/mL)	8	5	13
Grade 2 (>2<10 ng/mL)	0	2	2
Grade 3 (>10 ng/mL)	7	4	11
CRP mg/L (Median range)	31 (0-323)	28 (6-320)	28 (0-323)
White cell count (x 10^9 cells/L) Mean (SD)	14.4 (7.9)	12.2 (6)	13.2 (7)

Patient clinical information**:**

The majority of patients, **65% (n=32**) were neonates from the nursery ward or neonatal high care. The remainder of patients were from the paediatric resuscitation unit **(18%; n=9)** and general medical paediatric wards **(14%; n=7)** with 1 patient from intensive care unit.

Seven children presented with features suggestive of possible meningitis which included bulging fontanelle, fisting and meningism. Fever was described in clinical notes for less <5% of patients

CSF culture was performed on 42% (n=20) of patients with positive results in 4 individuals. Blood culture was performed on 47% (n=22), with positive results in more than sixty percent (n=14) patients. Organisms identified on positive culture results are summarized in **Table 2** below

Table 2 Organisms identified in culture

	Organisms identified
CSF Culture	➢ Staphylococcus aureus
	➢ Cryptococcus neoformans
	➢ Klebsiella pneumoniae
	➢ Actinobacteria baumanni
Blood Culture	➢ Staphylococcus aureus
	➢ Staphylococcus epidermis

Correlation of semi-quantitative PCT with CRP and other markers:

Kruskal Wallis analysis was used to the describe the relationship between the categorical variable of semi-quantitative PCT results with continuous variable of serum CRP results. Analysis revealed a statistically significant (p=0.035) difference of median CRP levels across different categories of PCT levels, with higher values associated with PCT categories associated with greater degrees of bacterial infection i.e. category 3 (PCT>2 ng/mL) and category 4 (PCT>10 ng/mL)

Kruskal Wallis analysis between absolute white cell counts and semi-quantitative PCT results showed no significant trend between categories of PCT results and WCC values (p=0.7)

Analysis between serum CRP levels and WCC showed no correlation between the two set of data with Spearman correlation of 0.017

Semi-quantitative PCT and culture results**:**

For analysis CSF and blood culture were combined. **Table 3** depicts the paired results for culture and PCT categorization. Kappa statistical analysis was performed to determine agreement between positive and negative culture results with positive (categories 1,2 and 3) and negative (category 0) semi-quantitative PCT results. Kappa statistic indicated poor observed agreement of 53.13% (acceptable would be considered >75%)

Table 3 Paired results for culture and PCT categorization

	PCT Categorization			
Category	**0**	**1**	**2**	**3**
Culture Positive	10	4	2	2
Culture Negative	7	5	1	1

Notes :PCT categorization 0=<0.5 ng/mL; 1=>0.5≤2 ng/mL; 2=>2 ≤10 ng/mL;3=>10 ng/mL)

In order to further analyse relationship between the semi-quantitative PCT results, CRP values and culture results. CRP was categorised into two groups Group 1 < 50 mg/L and Group 2 ≥50 mg/L. The value of 50 mg/L was chosen in view of previous reports, indicating this was the best cut off for the diagnosis of sepsis (sensitivity 98.5% and specificity 75%) [15]

Table 4. Paired results of CRP categoristion with culture results and semi-quantitative PCT categorization

	CRP Categorisation			
	Group 1 (< 50 mg/L)	**Group 2 (≥50 mg/L)**		
Culture Positive	11	9		
Culture Negative	9	6		
	Semiquantative PCT (SPCT) Categorisation			
PCT Category	**0**	**1**	**2**	**3**
Group 2 CRP (≥50 mg/L)	5	4	2	7

Notes:Semi-quantitative PCT= SPCT

PCT categorization 0=<0.5 ng/mL; 1=>0.5≤2 ng/mL; 2=>2 ≤10 ng/mL;3=>10 ng/mL)

Kappa statistical analysis was performed to determine agreement between positive and negative culture results with categories CRP group 1 and 2. Kappa statistic also showed poor observed agreement of 51.43%

Review of the suspected meningitis group:

With regards to patients with suspected CSF infection as defined by testing request for CSF chemistry, microscopy and culture and clinical chart review a total of 18 patients were identified. The characteristics of these patients are summarized in Appendix1 of the paper. Of these 18 patients, 9 were male, and 9 were female. Age range was from 0.03 months to 118.9 months (median = 0.685 months).

Patients with clinical features suggestive of meningitis were n=5(27.8%), while those with positive culture results were n=4 (22.2%). Symptomatic patients from those who were culture positive were n=1(25%).

Patients with high CSF protein results were n=9(50%), while n=2(22.2%) of those with high CSF protein results, also had positive CSF culture results. CSF microscopy findings showed that n=1(25%) of total CSF culture positive results showed predominantly polymorphonuclear cells, n=1(25%) showed no cells, n=1(25%) had no microscopy done, and n=1(25%) had a few lymphocytes. (see supplemental data for Table summarizing this data for all patients)

On chart review it was noted that most of this group where placed on multiple antibiotic agents irrespective of culture findings.

Discussion

This is the first study to the authors knowledge to describe the utility of semi-quantitative PCT testing in an African paediatric population. This assay would be perfectly suited for a resource constrained environment as it requires no special equipment or technical expertise to perform and is less costly than the formal assay. It is used primarily for sepsis diagnosis but not for monitoring therapeutic follow-up. This is evidenced in our study findings, where serial measurements were done for less than 25% of the patients reviewed, with only n= 11 having more than one result during the admission period.

As would be expected the semi-quantitative PCT results correlated well with the serum CRP levels and showed significant correlation with extent of rise in CRP. However no correlation was seen with white cell count, which has been shown in previous studies to be less useful than CRP for detection of bacterial infection. [16] Concordance of the semi-quantitative PCT results with the gold standard of culture was poor. This could be due to the following reasons: the small number of available culture results, the presence of possible contamination of culture specimen ; the small number of study cohort as well as the small number of patients with semi-quantitative PCT results in category 2 and above (n=6) (which is more suggestive of bacterial infection than category 1). Both CRP and semi-quantitative PCT agreement with culture results demonstrated a kappa statistic of less than the minimum acceptable of 75% (with CRP slightly worse at 51% versus PCT kappa statistic value of 53%)

One of the major limitations of the study was the small sample size, in particularly for the subset group with suspected meningitis. This resulted in difficulty drawing any definitive conclusions from the data extracted including not being able to define cutoffs for semiquantitative PCT that would be indicative of bacterial meningitis. Additionally, possible contamination of blood culture specimens for approximately nine of the samples further reduced numbers for examining sensitivity and specificity of the semi-quantitative PCT against the gold standard of culture. It was also noted that of those patients with positive CSF culture results, only 25% were symptomatic. This is supportive of studies that have found that clinical signs of meningism are not reliable in the paediatric group, and a high index of suspicion is needed. [17]

Confounders such as possible CSF and blood culture contamination and antibiotic use possibly affected culture results. Previous studies have shown that antibiotic use can affect confirmatory culture testing. [17]

Another limitation was the lack of compete medical records for some of the patients and lack of further investigations such as viral serology for exclusion of a viral meningitis. Due to the retrospective nature of this study semi-quantitative PCT analysis was not paired with formal PCT values to assess this correlation. However previous studies (including in house study performed, data not published) have confirmed good correlation with semi-quantitative and formal PCT assessment. [18]

Conclusion:

The finding of the current study does not demonstrate added benefit of use of semi-quantitative PCT over traditional CRP. Further studies in larger cohorts will need to be done to examine utility of the semi-quantitative PCT test for early diagnosis of specific bacterial infections including meningitis and pneumonia in this specific population.

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Manuscript Appendices:

Appendix 1: The characteristics of patients with suspected bacterial meningitis

Patient	Age (months)	Gender	Clinical symptoms	PCT	CRP	CSF Total protein	CSF glucose	CSF Microscopy	CSF Culture	Antibiotics used
1	3.5	F	No history	<0.5	28	0.62	3.2	10poly, 4 lymphocyte	Positive S. aureus	Not documented
2	3.8	M	Bulging fontanelle ;increased tone	<0.5	8	1.57	2.6	1 neutroph, 1 lymph	Negative	Augmentin,gentamicin, erythromycin, imipenen
3	3.8	M	Bulging fontanelle ,increased tone	<0.5	15	0.13	3.2	No cells	Negative	Augmentin, gentamycin, erythromycin
4	14.6	M	Meningism present	<0.5	152	ND	ND	ND	Positive Cryptococcus	Bactrim, cloxacillin, Bactrim,PZA, rifampicin, ISZ, ethambutol
5	23,5	M	No history	>0.5	89	0.14	3.4	2poly	Negative	Tazocin, ciprobay, flucloxacillin, imipenem
6	118.9	F	No history	>10	320	0.27	2.7	No cells	Negative	Not documented
7	0.07	F	No Meningism	>10	12	1.81	4.9	8poly,32lymph, >1000 eythrocytes	Negative	Gentamycin, solpen, tazocin, amikacin, imipenem
8	0.33	M	No Meningism	>10	58	0.79	6.4	0poly, 2lymph	Negative	Gentamicin, solpen
9	0.8	M	No Meningism	<0.5	25	ND	ND	Scanty poly and lymph	Negative	Amikacin, tazocin ciproflox, fluconazole, gentamicin, solpen
10	0.4	F	No Meningism	>10	28	1.88	4.5	ND	Negative	Gentamicin, ampicillin, vancomycin, imipenem
11	0.17	F	No history	<0.5	7	0.92	2.7	0 neutrophils, 0 lymphs	Positive Klebsiella pneumonia	Not documented

Table continued **Patient**	**Age (months)**	**Gender**	**Clinical symptoms**	**PCT**	**CRP**	**CSF Total protein**	**CSF glucose**	**CSF Microscopy**	**CSF Culture**	**Antibiotics used**
12	0.7	F	Jittery	<0.5	165	0.59	6.5	0 poly, 0 lymph	Negative	Solpen, imipenem, vancomycin, tazocin
13	0.9	M	No Meningism	<0.5	14	0.83	5.8	0 cells	Negative	Imipenem, clarithromycin, tazocin, amikacin, ampicillin, flagyl
14	0.3	M	No history	>0.5	130	2.42	8.5	0 poly, 2lymph, 70 erythrocytes	Positive Acinebacter	Not documented
15	0.3	M	No history	>10	72	0.59	4.4	0 cells	Negative	Not documented
16	0.03	F	Decreased tone	>0.5	6	1.14	10	Scanty leucocytes	Negative	Solpen, gentamicin, amikacin
17	1.5	F	No history	>2	160	1.5	5	0 cells	Negative	Not documented
18	0.5	F	No Meningism	>0.5	7	1.48	1.6	ND	Negative	Amikacin, ciprobay, gentamicin

Appendix 1: The final Study Protocol (Include the final protocol which was given full approval by Brec and/or the postgrad office)

MMED Research Protocol

Title: Comparison of Semi quantitative Procalcitonin versus C-Reactive Protein for the diagnosis of bacterial meningitis in children

Dr Unathi Ngxamngxa

213573224

Title of study

Comparison of semi quantitative Procalcitonin versus C-Reactive Protein for the diagnosis of bacterial meningitis in children

Aim

To compare the ability of serum procalcitonin (semi quantitative PCT) versus C-reactive protein (CRP), to differentiate between bacterial and viral meningitis in a paediatric population.

Objectives

a) Determine correlation of semi quantitative PCT versus CRP results to microscopic and culture evidence of bacterial meningitis

b) Determine whether procalcitonin measured alone, or used in conjunction with c-reactive protein is a better predictor/marker of bacterial meningitis infection in paediatric patients

c) Determine possible diagnostic cut off levels for semi quantitative PCT and CRP for bacterial meningitis

d) Determine correlation of PCT and CRP with other inflammatory markers White cell count and erythrocyte sedimentation rate (ESR) in the presence of bacterial meningitis.

Summary of proposed study

The high morbidity and mortality rate due to meningitis in the paediatric population, makes it imperative that diagnosis is made promptly so as to initiate the appropriate therapy. PCT and CRP are important adjunctive tests to gram staining and culture of CSF and blood samples in making the diagnosis.
This project aims to compare how these two biomarkers compare when used to differentiate between bacterial and viral meningitis. Although PCT and CRP have been studied extensively regarding their diagnostic and prognostic value, few of these studies have been performed in

Africa and no studies have been done looking at the utilization of semi quantitative PCT in this setting. Both tests are frequently requested by clinicians, and it is important to determine whether this practice is both evidence based, and cost effective.
The study will look at the diagnostic value of semiquantitative PCT and CRP results of paediatric patients with meningitis from one of the local hospitals, King Edward Hospital.

Background and literature review

The diagnosis of bacterial infections is important, as they are a significant cause of morbidity and mortality.[1] The worldwide annual incidence is 1.2 million cases/year, with a 135 000 deaths.[2] In the South African paediatric population the yearly incidence of bacterial meningitis is 40 per 100 000 in the <1 year age group, and 7 per 100 000 in the 1-4 year age group[3] In children it is often difficult to determine if the aetiology of meningitis is viral or bacterial. Early diagnosis of bacterial meningitis is critical in order to initiate appropriate therapy and to prevent the unnecessary and widespread use of antibiotics in cases of viral meningitis [1,4]

Distinguishing bacterial and viral meningitis early could also help to reduce hospital admissions. Use of clinical criteria, Gram staining, and bacterial antigen testing of CSF as well as the inflammatory markers in the blood (CRP level, white blood cell count [WBC], and neutrophil count) or CSF (protein level, glucose level, WBC count, and neutrophil count) used alone do not offer 100% sensitivity with high specificity for distinguishing bacterial and viral meningitis. Results of CSF culture may take a number of days and detection of viral agents using PCR is not readily available in most centers. [5]

The diagnostic challenge posed by similar clinical features of bacterial and viral infections makes the role of biomarkers such as PCT critical in identifying bacterial infections. [5]

In young children the clinical features of meningitis may differ according to age, and are often not the classical signs of meningitis found in older children and adults. This poses a diagnostic challenge, and makes the role of tests such as CSF chemistry and bacterial culture, blood culture and serum PCT or CRP and peripheral white cell count imperative in making the diagnosis. [3]

The bacterial meningitis score is useful in differentiating bacterial from viral meningitis. The score is negative when the following are absent; CSF gram stain, CSF neutrophil count ≥ 1000

cells/mm^3 , CSF protein ≥0.8 g/l, peripheral blood absolute neutrophil count ≥ 10 000 cell/mm^3 and history of seziures peri-onset of presentation. [3]. According to Boyles et al, when the BMS is negative, the negative predictive value is 99.9%. The limitation for the score is that it is not validated to use in certain patient groups; such as infants< 2months and immunocompromised patients amongst others. [3]

Procalcitonin is a 116 amino acid polypeptide which is a precursor of calcitonin, which is synthesized in the thyroid C cells. Calcitonin inhibits bone resorption during hypercalcaemic states. [6,7] Procalcitonin became a useful biomarker for sepsis in 1993. [9]

PCT is an immunologically active protein which has tightly regulated levels and hence differs from other inflammatory molecules such as cytokines, acute phase proteins and leucocyte surface markers. It is a good marker of infections secondary to bacterial infections and is produced by many tissues. [8]

Local or systemic infection results in PCT production in response to monocytes produced. The biological effects of PCT include chemokine effects, stimulating release of proinflammatory cytokines and modulating vascular contraction. [8]It was been demonstrated that serum PCT levels increase during the course of bacterial, parasitic, or fungal infections, but remain normal or slightly increase in viral infections and inflammatory reactions that are not infectious. [5] PCT differs from other markers of sepsis such as CRP, lactate and cytokines in that it is more specific for sepsis, and can also indicate the severity of sepsis. [9]

PCT methods are immunoassay based. They are quantitative or semi-quantitative, and are measured in serum or plasma. Methods used to measure PCT need to have high sensitivity and have detection limits as low as 0.02 ng/ml. [8 Semiquantitative PCT methods have been shown to have 93.94% specificity and 87.23% specificity for severe sepsis. [10] According to a study by Oh J et al, these semiquantitative methods are fast and effective, and are useful to guide antibiotic treatment. Both these methods are available in South African laboratories with quantitative analysis found in larger academic centres. However Procalcitonin tests costs are significantly higher than that of CRP. Semi quantitative methods are cheaper, more readily available and do not require expensive instrumentation.

C-reactive protein was discovered in 1930 by Tillet and Francis, who noted that the protein formed a complex with pneumococcal C-Polysaccharide during pneumonia infection. [11]
CRP is a sensitive acute-phase reactant for inflammation which is made in the liver. It activates the classical complement pathway. [11]

CRP concentrations increase in response to infection, trauma, surgery and chronic inflammatory conditions. Mild inflammatory conditions and viral infections cause lower CRP production than bacterial infections, with the highest concentrations being found in severe bacterial infections. [11]

CRP methods include immunoturbidimetric and Fluorescence labeled ELISA methods, which are commonly used. These automated methods offer high sensitivity and specificity. Latex agglutination methods are used for semiquantitative testing and are available as point of care tests[11]

PCT has shown better sensitivity (88%) and specificity (81%) compared to CRP with a sensitivity of 75% and specificity of 67% in the diagnosis of bacterial infection. [1]
PCT also has an added advantage of being elevated within 2-4 hours, while CRP rises within 12-24 hours post inflammation. PCT with a half-life of 24 hours, returns to normal levels far earlier than CRP, which may remain high for up to 7 days post recovery. Nargis et al demonstrated that the use of PCT reduces the number of days for which antibiotics are taken, and results in a reduced hospital admission. [7]

The indiscriminate increase in CRP due to viral or bacterial infections, makes PCT a superior biomarker in order to differentiate viral from bacterial meningitis. [2] Another advantage of measuring PCT instead of CRP, is that use of anti-inflammatory drugs, steroids and inflammatory conditions such as inflammatory bowel disease, cannot affect its concentration. The study by Vikse et al mentioned that the limitations of PCT in the diagnosis of bacterial meningitis in adults, included its decreased concentration when antibiotics are used, and an increase in other bacterial infections such as pneumonia and sepsis.

A study by Alkholi et al showed that PCT levels were significantly higher in patients with bacterial meningitis (mean, 24.8 ng/ml) compared to patients with viral meningitis (mean, 0.3 ng/ml) (P<0.001). The aim of the present study was to determine the role of serum PCT levels in the early diagnosis of bacterial meningitis and to document their efficacy in the differential

diagnosis of viral and bacterial meningitis. The study also demonstrated that PCT levels in bacterial meningitis group decreased sooner in those children after treatment initiation. Guidelines from Boyles et al recommend that antibiotic treatment may be stopped when CRP is <20mg/l, as it has a negative predictive value for bacterial meningitis of 99%, and PCT <0.5 ng/ml, as it has a negative predictive value of 99% for bacterial meningitis.

Whilst both PCT and CRP testing are readily available in most labs in South Africa, there are however few studies that have investigated their diagnostic and prognostic value in early detection of bacterial meningitis in Africa.

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Study design

A retrospective, observational, analytical study

Study population and location

Children aged between 4 months and 12 years admitted with meningitis at King Edward Hospital.

Sampling strategy

Procalcitonin and CRP results from paediatric wards requested and performed over the period of the study April 2013- April 2016 will be extracted from the central data warehouse.
Purposive sampling will be used.

Chart review of identified patients will be performed using patient folders.

Sample size

Based on a previous study, and after consultation with the statistician, a sample size of 40 was decided upon. (refer to study Alkholi et al). The prevalence of bacterial meningitis was found to be 18 cases in the whole of 2015 at the study hospital. Based on this a 3 year study period with a sample size of 40 cases was chosen.

Inclusion criteria

- Paediatric patients between 4 months and 12 years admitted with suspected meningitis during the period from April 2013 – April 2016 that have had procalcitonin and CRP testing performed

Exclusion criteria

- Patients with documented infections in addition to meningitis

Data collection strategy and methods

Procalcitonin and CRP results from paediatric wards requested and performed over the period of the study will be extracted from our laboratory information system central data warehouse. The data collection tool will be the printout of patient details and data from the central data wharehouse.

Chart review of identified patients will be performed using the hospital information system. Patients with meningitis will be identified as those diagnosed based on evaluation of history, physical examination, CSF laboratory findings, identification of bacterial agents in CSF, Gram staining, and cultures. Bacterial meningitis will be defined according to CSF laboratory findings (increased protein ≥2 g/l, decreased glucose ratio ≤0.4, and leukocyte count ≥1500 × 10/l and polymorph nuclear leukocyte domination), identification of bacterial agents in Gram staining, and/or positive bacterial culture. Viral meningitis will be defined as viral if the viral culture, serological testing, pleocytosis, or reverse transcriptase polymerase chain reactions were positive, and the bacterial culture was negative

Statistical analyses

Data will be entered into SPSS version 24 (Statistical Packages for the Social Sciences) for analysis. A p value <0.05 will be considered as statistically significant. A descriptive statistical analysis of the data (means, standard deviations, ranges, frequencies and percentages, etc.) will initially be conducted prior to inferential analysis.

Differences between groups in continuous variables will be tested for significance with the Mann-Whitney test. Differences in frequencies of findings between groups will be analyzed by Fischer's exact test. Sensitivity and specificity will be derived from the receiver operating characteristic (ROC) curve, and area under the ROC curve. The chi-square test of association will be used to assess any associations between categorical variables. Kappa statistics will be used to compare the level of agreement between tests.

Study period

Results and data from April 2013- April 2016.

Study Design: Retrospective, observational, cross sectional study

Limitations of the study:

- This is a retrospective data review and therefore, lack of data may limit the number of data subjects for analysis.

Ethical considerations:

There will be no patient interaction as this is a retrospective chart review. The risks are therefore minimal. This study will have no impact on patients and will not affect their treatment or outcome. It is a non-invasive study and does not require any extra blood sampling. Patient confidentiality will be maintained at all times. Data will be collected on a password protected computer and the primary investigator will be the only person with access to it. Patients will be identified by hospital numbers and their identities will not be revealed.

The benefit of the study is that the clinical data acquired from these charts has the potential to guide patient testing for infective markers to allow for increased cost effectiveness and sensitivity in the diagnosis of bacterial meningitis in children

Supervision and collaboration:

This research project will be performed under the supervision of Dr Verena Gounden and Dr Ravindra Sirkar , consultant chemical pathologists at the Department of Chemical Pathology UKZN.

Appendix 2: The Guidelines for Authorship for the Journal selected for submission of the manuscript

Include the author guidelines in order that the examiner can assess adherence to the journal requirements.

ISSN 0256-9574 ***printed version***
ISSN 2078-5135 ***online version***

INSTRUCTIONS TO AUTHORS

Scope and policies

The *SAMJ* is a monthly, peer–reviewed, internationally indexed, general medical journal publishing leading research impacting clinical care in Africa. The Journal is not limited to articles that have 'general medical content', but is intending to capture the spectrum of medical and health sciences, grouped by relevance to the country's burden of disease. This will include research in the social sciences and economics that is relevant to the medical issues around our burden of disease.

The journal carries research articles and letters, editorials, clinical practice and other medical articles and personal opinion, South African health–related news, obituaries, general correspondence, and classified advertisements (refer to the section policies for further information).

Conflict of interest

Conflicts of interest can derive from any kind of relationship or association that may influence authors' or reviewers' opinions about the subject matter of a paper. The existence of a conflict – whether actual, perceived or potential – does not preclude publication of an article. However, we aim to ensure that, in such cases, readers have all the information they need to enable them to make an informed assessment about a

publication's message and conclusions. We require that both authors and reviewers declare all sources of support for their research, any personal or financial relationships (including honoraria, speaking fees, gifts received, etc) with relevant individuals or organisations connected to the topic of the paper, and any association with a product or subject that may constitute a real, perceived or potential conflict of interest. If you are unsure whether a specific relationship constitutes a conflict, please contact the editorial team for advice. If a conflict remains undisclosed and is later brought to the attention of the editorial team, it will be considered a serious issue prompting an investigation with the possibility of retraction.

Manuscripts preparation

Preparing an article for anonymous review

To ensure a fair and unbiased review process, all submissions are to include an anonymised version of the manuscript. The exceptions to this are Correspondence, Book reviews and Obituary submissions.

Submitting a manuscript that needs additional blinding can slow down your review process, so please be sure to follow these simple guidelines as much as possible:

- An anonymous version should not contain any author, affiliation or particular institutional details that will enable identification.
- Please remove title page, acknowledgements, contact details, funding grants to a named person, and any running headers of author names.
- Mask self–citations by referring to your own work in third person.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word or RTF document format. Text must be single–spaced, in 12–point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).

- Please make your article concise, even if it is below the word limit.
- Qualifications, full affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and °C), e.g. '40 kg' and '20 cm' but '50%' and '19°C'.
- Please be sure to insert proper symbols e.g. ɲ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand–units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format – this is the only exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.
- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.
  **NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.
- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
- Use the latest approved gene or protein symbol as appropriate:
  - Human Gene Mapping Workshop (HGMW): genetic notations and symbols
  - HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
  - OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions

- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424–433: standard human pedigree nomenclature.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain). –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
- Embed/include each table in the manuscript Word file – do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell–based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.

- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

    - ***Do not***: Use [Enter] within a row to make 'new rows':

    - *Rather*:
      Each row of data must have its own proper row:

    - ***Do not***: use separate columns for n and %:

    - *Rather*:
      Combine into one column, n (%):

    - ***Do not***: have overlapping categories, e.g.:

    - *Rather*:
      Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and* ***not*** *with the use of reference manager software. Endnotes must not be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4–6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.

- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215–1217 **not** 1215–17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link).
  Authors are encouraged to use the DOI lookup service offered by CrossRef:
    - On the Crossref homepage, paste the article title into the 'Metadata search' box.
    - Look for the correct, matching article in the list of results.
    - Click Actions > Cite
    - Alongside 'url =' copy the URL between { }.
    - Provide as follows, e.g.:
      https://doi.org/10.7196/07294.937.98x

  Some examples:

- Journal references: Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355.
  http://dx.doi.org/10.1000/hgjr.182
- Book references: Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96–101.
- Chapter/section in a book: Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457–472.
- Internet references: World Health Organization. The World Health Report 2002 – Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002.
  http://www.who.int/whr/2002 (accessed 16 January 2010).

- Legal references

    - Government Gazettes:
      National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.
      In this example, 17507 is the Gazette Number.

This is followed by :1514 – this is the notice number in this Gazette.

- Provincial Gazettes:
  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:
  South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:
  South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:
  South Africa. Traditional Health Practitioners Bill, No. B66B–2003, 2006.

- Green/white papers:
  South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:
  Rex v Jopp and Another 1949 (4) SA 11 (N)
  Rex v Jopp and Another: Name of the parties concerned
  1949: Date of decision (or when the case was heard)
  (4): Volume number
  SA: SA Law Reports
  11: Page or section number
  (N): In this case Natal – where the case was

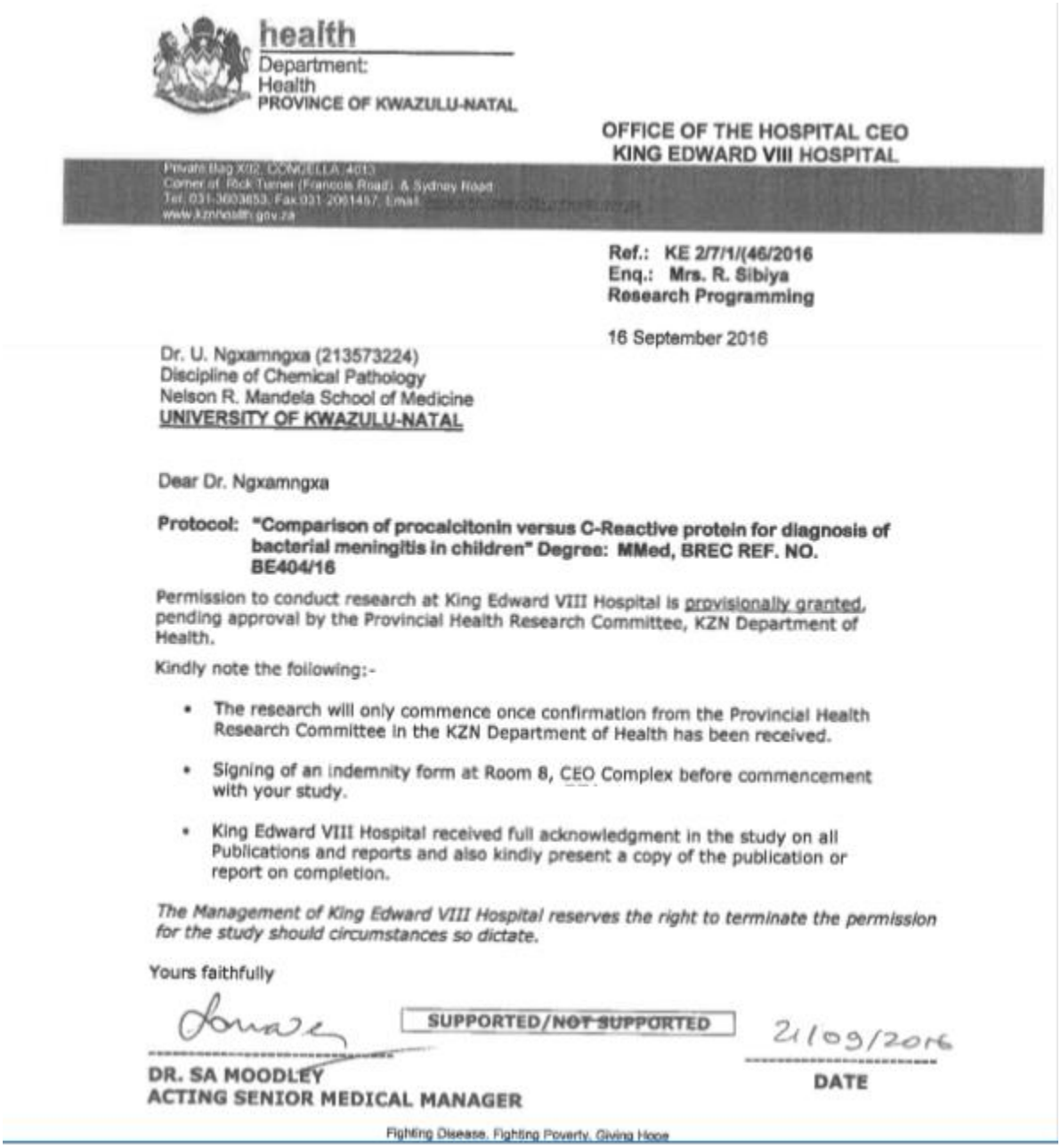
heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

- *Other references* (e.g. reports) should follow the same format: Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

Appendix 3: Ethical approvals

Included hospital and provincial approvals as well as the BREC approval (or waiver if appropriate).



health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII HOSPITAL

Private Bag X02, CONGELLA, 4013
Corner of Rick Turner (Francois Road) & Sydney Road
Tel: 031-3603653, Fax 031 2061457, Email
www.kznhealth.gov.za

Ref.: KE 2/7/1/(46/2016
Enq.: Mrs. R. Sibiya
Research Programming

16 September 2016

Dr. U. Ngxamngxa (213573224)
Discipline of Chemical Pathology
Nelson R. Mandela School of Medicine
UNIVERSITY OF KWAZULU-NATAL

Dear Dr. Ngxamngxa

Protocol: "Comparison of procalcitonin versus C-Reactive protein for diagnosis of bacterial meningitis in children" Degree: MMed, BREC REF. NO. BE404/16

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

SUPPORTED/~~NOT SUPPORTED~~ 21/09/2016

DR. SA MOODLEY
ACTING SENIOR MEDICAL MANAGER

DATE

Fighting Disease, Fighting Poverty, Giving Hope

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

14 March 2019

Dr U Ngxamngxa (213573224)
Discipline of Chemical Pathology
School of Laboratory Medicine and Medical Sciences
ungxamngxa@gmail.com

Title: Comparison of procalcitonin versus C-Reactive protein for diagnosis of bacterial meningitis in children.
Degree: MMed
BREC REF NO: BE404/16

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	11 January 2019
Expiration of Ethical Approval:	10 January 2020

I wish to advise you that your application for Recertification received on 28 February 2019 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 April 2019.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: supervisor: verenagounden@yahoo.com
cc postgraduate administrator: dudhra.jhp@ukzn.ac.za

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

330 Langalibalele street,
Private Bag X9051 PMB, 3200
Tel: 033 395 2805/3189/3123 Fax: 033 394 3782
Email: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management (HKRM)

Reference: HRKM404/16
KZ_2016RP43_493

02 December 2016

Dear Dr. U Ngxamngxa
(University of KwaZulu-Natal)

Subject: Approval of a Research Proposal

1. The research proposal titled **'Comparison of Semi quantitative Procalcitonin versus C-Reactive Protein for the diagnosis of bacterial meningitis in children'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at King Edward VIII Hospital.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 06/12/16

Appendix 4: Data collection tools (for example)

Not applicable

Appendix 5: Raw data

DOA	Patient ur	Patient file number	Twin		Age raw	Age (months)
14/07/2013	2	15152/13			28days	0.93
01/10/2014	206	65542/14			1month7days	1.23
No file	7033	2173/15			1yr17days	12.57
No file	7037	24320/15			3mnths15days	3.50
13/11/2015	7058	2641/15	twin 1		3mnths23days	3.77
13/11/2015	7475	2642/15	twin 2		3mnths23days	3.77
16/08/2016	7487	18951/16			2mnth23days	2.77
16/09/2014	7498	7396/14			1yr2mnths17days	14.57
No file	7505	71378/15			1yr11mnths15day	23.50
27/05/2015	7515	12548/15			1yr0months0days	12.00
Not documented	7516	27773/15			3mnths17days	3.57
	7533	2208/16			7yrs6mnths16day	90.53
	7536	4242/14			9yrs12days	108.40
	7538	10556/15			9yrs7days	108.63
	7541	6973/15			9yrs10mnths26da	118.87
22/12/2015	7559	29617/15			11yrs2days	132.07
	15	50191			No age	
31/10/2014	16	513771			2days	0.07
02/11/2014	17	513817			10days	0.33
	19	600687			4days	0.13
	21	515211			2months5days	2.17
	22	600648			2mnths12days	2.40
	23	603462			7days	0.23
	24	603704			12 days	0.40
	26	603686			3 days	0.10
	27	603443			13 days	0.43
	28	604495			20 days	0.67
06/05/2015	29	604589			24 days	0.80
	30	605380			11days	0.37
	31	605854			No age	0.03
Not documented	32	605852			5days	0.17
	37	606457			1mnth14days	1.47
02/06/2015	38	606267			21 days	0.70
	39	60694			4 days	0.13
Not documented	40	606327			9 days	0.30
	42	606144			27days	0.90
	44	607158			6days	0.20
	45	605672			1mnth8days	1.27
	46	606406			9days	0.30
Not documented	48	607336			9 days	0.30
	49	607569			9 days	0.30
	50	606941			1day	0.03
	53	610479			1day	0.03
	54	610339			6days	0.20
	55	612371			6days	0.20
	56	612741			2days	0.07
	61	613527			1mnth15days	1.50
	62	70199			6days	0.20
12/01/2016	63	700286			16days	0.53

Gender (M/F)	Clinical picture (meningitis/ premature)	Clin catergorisation	Fever(Y/N)	Ward	PCT	PCT catergorisation
M	No meningism,ex prem	0	Y	PRU	>0.5	1
F	No meningism	0	Y	PRU	<0.5	0
M	No history	2		Med ward	<0.5	0
F	No history	2		PRU	<0.5	0
M	Bulg fontanelle, incr tone, LBW	1	N	Med ward	<0.5	0
M	Bulg fontanelle, incr tone, brisk ref, LBW	1	Y	Med ward	<0.5	0
F	No meningism, fever present	1	Y	PRU	>10	3
M	Irritable with meningism	1	Y	Med ward<0	<0.5	0
M	No history	2		PRU	>0.5	1
M	No features of meningitis	0	Y	Med ward	<0.5	0
F	No features of meningitis	0	Y	PRU	>0.5	1
F	No history	2		Med ward	>0.5	1
M	No history	2		Med ward	<0.5	0
M	No history	2		PRU	>10	3
F	No history	2		PRU	>10	3
M	No meningism	0	Y	PRU	<0.5	0
M	No history	2		Nursey	>10	3
F	No meningism	0	N	Nursey	>10	3
M	No meningism	0	N	Nursey	>10	3
M	No history	2		Nursey	<0.5	0
F	No history	2		Nursey	>2	2
F	No history	2		Nursey	<0.5	0
M	No history	2		Nursey	>0.5	1
M	No history	2		Nursey	>10	3
M	No history	2		Nursey	>10	3
F	No meningism	0	N	Nursey	<0.5	0
M	No meningism	0	N	Nursey	<0.5	0
M	No meningism	0	N	Nursey	<0.5	0
F	No meningism	0	N	Nursey	>10	3
M	No meningism	0	N	Nursey	>2	2
F	No history	2		Nursey	<0.5	0
F	No history	0		Nursey	<0.5	0
F	Jittery	1	N	ICU	<0.5	0
M	No history	2		Nursey	>0.5	1
M	No history	2		Nursey	>0.5	1
F	No meningism	0	Y	Nursey	<0.5	0
M	No history	2		Nursey	>10	3
M	No meningism	0	N	Nursey	<0.5	0
M	No history	2		Nursey	>10	3
M	No history	2		Nursey	>0.5	1
M	No history	2		Nursey	>10	3
M	No history	2		Nursey	>0.5	1
F	Decr tone	1	N	Nursey	>0.5	1
F	Fisting, incr tone	1	N	Nursey	>0.5	1
M	No history	2		Nursey	>0.5	1
M	No history	2		Nursey	<0.5	0
F	No history	2		Nursey	>2	2
M	Abnormal neurology	1	N	Nursey	<0.5	0
F	No menigism	0	Y	Nursey	>0.5	1

CRP mg/L	WCC	CSF TP		CSF TP	CSF glu	CSFcl	CSF micro	CSF Culture pos /neg (number	CSF Culture categorisation
27	15.43						0poly, 4lymph,260erythro and xantho trace	Negative	1
13	10.05							Not done	0
135	8.3							Not done	0
28	No result	0.62		High	3.2	118	10Poly,4lymph	Positive	2
8	14.19	1.57		High	2.6	120	1+neutroph, 1+lymph	Negative	1
15	12.58	0.13		Low	3.2	117	0poly,0lymph	Negative	1
39	10							Not done	0
152	19.6							Positive	2
89	2.8	0.14		low	3.4	115	2poly	Negative	1
33	13.35							Not done	0
31	7.7							Not done	0
188	10.3							Not done	0
133	1.84							Not done	0
323	18.6							Not done	0
320	No result	0.27		normal	2.7	122	no cells	Negative	1
9	18.08							Not done	0
11	clotted							Not done	0
12	8.67	1.81		High	4.9	121	8poly, 32 lymph,>1000erythrocytes	Negative	1
58	3.26	0.79		Normal	6.4	125	0poly, 2lymph	Negative	1
75	17.14							Not done	0
166	19.71							Not done	0
18	25.43							Not done	0
0	insuitable							Not done	0
9	24.4							Not done	0
125	15.54							Not done	0
8	6.59							Not done	0
17	2.35							Not done	0
25	14.84						scanty poly&lymph	Negative	1
28	14.25	1.88		High	4.5	123		Negative	1
31	7.06							Not done	0
7	3.74	0.92		High	2.7	110	0 neutr, 0lymph	Positive	2
6	8.5							Not done	0
165	13.35	0.59		Normal	6.5	109	0poly,0lymph	Negative	1
7	no result							Not done	0
5	7.22							Not done	0
14	10.18	0.83		High	5.8	114	0 cells	Negative	1
54	17.19							Not done	0
40	14.6							Not done	0
175	clotted							Not done	0
130	15.22	2.42		High	8.5	109		Positive	2
72	insuff	0.59		Normal	4.4	122	0 cells	Negative	1
27	25.56							Not done	0
6	22.36	1.14		High	10	119	scanty leucocytes	Negative	1
67	7.17							Not done	0
29	26.3							Not done	0
16	7							Not done	0
160	11.91	1.5		High	5	118	0poly,0lymph	Negative	1
6	21.5							Negative	1
7	16.5	1.48		High	1.6	120	clotted	Negative	1

No bacteria seen	Negative	1		Amikacin, tazocin, cefotaxime			
	Not done	0		No antibiotics			
	Positive	2	Staph aureus	Not known			
Staph Aureus(?contam	Positive	2	Stap aureus	Not known			
	Positive	2	Staph species(?contam	Augmentin,genta,erythromycin,imipenem			
	Positive	2	Gram positive cocci(?c	Augmentin,genta,erythromycin			
	Positive	2	Gram positive cocci(?c	Tazocin, amikacin			
Cryptococcus							
No culture	Negative	1	No growth				
	Positive	2	Staph aureus				
	Positive	2	Staph aureus				
	Negative	1	No growth				
	Negative	1	No growth				
	Not done	0		Not documented			
No growth				Not documented			
	Negative	1	No growth				
	Not done	0					
No growth							
No growth							
	Positive	2	gram positive cocci and bacilli(?contam)				
	Positive	2	Gram pos bacilli (?contam)				
	Negative	1	No growth				
	Positive	2	Staph species(?contam)				
	Positive	2	Gram positive cocci in clusters				
	Negative	1	No growth				
	Positive	2	Stah epidermidis(?contam)				
	Positive	2	Staph species(?contam)				
No growth							
				Antibiotics-Genta, ampicillin, vanco,imipenem			
Klebsiella pn							
	Not done	0					
	Not done	0					
No growth	Negative	1	No growth				
	Positive	2	Klebsiella pneumonia				
	Positive	2	Klebsiella&Staph				
	Not done	0					
Acinebacter							
No growth							
	Positive	2	Gram neg bacilli(Acinetobacter				
No growth							
Positive	Gram neg bacilli, acibacter						
	Negative	1					
	Positive	2	Staph species(?contamination)				
No growth							
No growth							
No bacteria							