The discriminative value of C-reactive protein levels in distinguishing between bacterial and viral pneumonia in children.

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Declaration

I, Sanjay Govind Lala, hereby declare that this research report is my own work and has not been presented for any degree of another university.

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16 September 1999

The work reported in this research report was performed in the Pneumococcal Unit of the South African Institute of Medical Research and the Department of Paediatrics and Child Health, Chris Hani-Baragwanath Hospital, Johannesburg

Dedicated to:

My father, Govind Lala (1935 -1977)

who laid the foundation for my future

My mother, Savita Lala

for allowing me to pursue my dreams without hindrance

My wife, Jessica

for her love and support

ABSTRACT

Background: CRP testing is routinely performed on children admitted to the Chris Hani-Baragwanath Hospital (Soweto, Johannesburg) with pneumonia. The CRP is used to distinguish between bacterial and viral pneumonia, assisting the clinician to judiciously prescribe antibiotics.

Objectives: 1. To evaluate whether the initial CRP measurement discriminates between bacterial and viral pneumonia. 2. To evaluate the effect of HIV infection on CRP responses in children with pneumonia. 3. To perform a costs analysis of routine CRP measurements in childhood pneumonia.

Design: Retrospective review of case records.

Results: This study analysed 570 children with pneumonia who were catergorised into four aetiological groups- 55 children had bacterial pneumonia, 145 viral pneumonia, 11 mixed pneumonia and in 359 children the aetiology was unknown. 244 (42.8%) children were co-infected with HIV and 186 (32.6%) children were malnourished. The median CRP value was significantly higher in bacterial pneumonia than in viral pneumonia or pneumonia of unknown aetiology (P <0.0001, Median test). Threshold CRP values of ≥10mg/L could distinguish between bacterial and viral pneumonia (P=0.0009, Fishers exact test). However, in HIV uninfected children, receiver-operating characteristic (ROC) curve plots showed that only 80% of bacterial infections could be predicted using threshold CRP values. A CRP value ≥10mg/L predicted all cases of bacterial pneumonia in HIV infected children. HIV infection did not affect CRP responses in children suffering from bacterial and/or viral pneumonia. Costs analysis suggests that routine CRP testing is expensive and outweighs the savings accrued by sparing the use of antibiotics in viral pneumonia.

Conclusions: In HIV uninfected children, the initial CRP does not detect 20% of children with bacterial pneumonia. In HIV infected children, other studies are needed to confirm whether a CRP value ≥10mg/L predicts all cases of bacterial pneumonia. Routine CRP testing is not recommended in children with pneumonia.

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1.0 INTRODUCTION

Pneumonia is a common problem in paediatric practice in the developing world. In such countries, pneumonia and diarrhoea are the leading causes of death among under-5-year olds, each accounting for approximately 19% of the 11.6 million deaths in 1995.[1]

A global WHO initiative, based on diagnosing acute pneumonia using clinical signs and instituting appropriate antibiotic therapy, has led to a reduction in acute respiratory infection-specific mortality of between 25% and 67%.[2]

Most cases of pneumonia are due to bacterial and/or viral agents.[3] This differentiation is important because bacterial pneumonia requires antibiotic therapy. Viral pneumonia is managed symptomatically and treatment of viral pneumonia with antibiotics promotes the development of antibiotic resistance. Several studies that investigated the aetiology of pneumonia in developing countries have implicated a bacterial cause in over 50% of cases, [4-7] and in some children bacterial infection may follow an initial viral infection.[8]

1.1 The diagnosis of pneumonia in children

The four major methods used to identify bacterial pathogens are (i) the culture of specimens including blood and lung aspirates, (ii) bacterial antigen detection tests, (iii) bacterial nucleic acid detection and (iv) bacterial antibody assays.[9] Lung puncture and culture of the aspirate probably has the highest yield in proving a bacterial aetiology. However, it is too invasive to be performed in routine practice and should only be performed in cases where lobar consolidation is present.[5,6,10-12] Nucleic acid detection of bacterial agents causing pneumonia has not been fully evaluated, thus it is limited to use in research settings presently.[6] Bacterial antigen detection tests and antibody assays have sensitivities and specificities that are too

low to be recommended for routine use.[13-15] The expense and delay in obtaining results (often days after antibiotic therapy has been started), are further limiting factors.[13]

Chest radiographs are often performed in children diagnosed with pneumonia. These radiographs are not helpful in distinguishing bacterial from viral pneumonia.[16-18] The pattern of the infiltrate seen on the chest radiograph may occasionally provide aetiologic clues, as in the case of lobar consolidation which suggests a bacterial aetiology. In addition, although alveolar infiltrates on chest radiographs are specific indicators of bacterial pneumonia, this feature lacks sensitivity. Interstitial infiltrates do not differentiate bacterial from viral pathogens.[18] Importantly, the decision regarding the initiation of antibiotic therapy cannot be based on radiographic criteria alone.

In practice, the confirmation of bacterial pneumonia is difficult and viral cultures are not routinely performed. Many children investigated for pneumonia have sterile bacterial and viral cultures. Bacterial pneumonia is a diagnostic challenge, and only verified by positive blood cultures in approximately 7% of cases.[13] Some children with pneumonia are co-infected with bacteria and viruses,[8] that contribute to the difficulty of separating bacterial from viral infections.

Faced with these difficulties, many investigators have measured various acute phase reactants in an attempt to differentiate bacterial from viral pneumonia. These reactants include the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP). Various cytokines, including interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-alpha) have been evaluated to determine if they can reliably predict bacterial infection.[19, 20] These latter measurements are limited to experimental studies presently, and they appear to hold no advantage

over CRP measurements.[20] ESR and CRP are the most widely performed investigations, which measure the acute phase response, in clinical practice.[20, 21]

Tillet and Francis discovered CRP in 1930, while investigating serological reactions in pneumonia. They noted that a non-type-specific somatic polysaccharide fraction of *Streptococcus pneumoniae*, which they designated fraction C, was precipitated by the sera of acutely ill patients. This precipitate disappeared rapidly as the patients improved.[22] The C-reactive protein (CRP) is an acute phase reactant synthesized by hepatocytes in response to various stimuli, including infection, trauma, surgery, burns, tissue infarction, various inflammatory conditions and advanced cancer.[20] CRP synthesis increases within 4 to 6 hours following the onset of inflammation or tissue injury, doubles every 8 hours thereafter, and peaks at 36 to 50 hours.[22] The half-life of CRP is thought to be 18 hours.[23] The normal range of CRP values in humans is regarded as 0 - 10mg/L.[22,23] Currently the most widely used technique for measuring CRP is nephelometry, which requires only 50µl of serum. An advantage of using CRP is that the results are generally rapidly available, usually within 15 - 30 minutes.[21]

CRP, a component of the innate immune system has two major biological roles. Firstly, it has a number of recognition capabilities, that is, it is able to bind to biologic substrates. Secondly, it has the capacity to activate the complement system and to modulate the function of leukocytes. A major function of CRP is its ability to bind to phosphocholine. Phosphocholine is present within the cell walls of bacteria, fungi and parasites, and is a component of eukaryotic cell membranes. Thus it is suggested that CRP plays a role in the clearance of exogenous infectious agents and endogenous damaged cells.[23]

CRP levels have been documented in many bacterial and viral infections. Generally CRP levels in acute invasive bacterial infections are elevated in the range of 150 to

300 mg/L. In most acute viral infections, CRP values tend to be much lower, <20 to 40 mg/L.[21] As many bacterial and viral infections commonly present with similar clinical syndromes (for example, pneumonia and gastroenteritis), CRP measurements may be useful in differentiating bacterial and viral infections. CRP determinations have been found to be useful in distinguishing bacterial meningitis from viral meningoencephalitis.[24] This has prompted several investigators to evaluate its usefulness in distinguishing between bacterial and viral pneumonia in children.

1.2 CRP measurements in children with pneumonia

One of the earlier studies that assessed the value of CRP testing in children with pneumonia concluded that the CRP was a better indicator of bacteraemia and lobar infiltrates then the ESR and WCC, it had the best predictive values and sensitivities for pneumonia of probable or proven bacterial aetiology. The CRP was performed as a rapid slide test and was reported as either positive (serum dilution of 1:50) or negative (serum dilution of 1:5 or less).[25]

Later studies, which quantified CRP measurements as newer techniques (turbidometry, ELISA) became available, also concluded that the CRP value was a useful test in the evaluation of children with pneumonia.[26, 27] Putto *et al.* prospectively studied 154 febrile children, who had been ill for more than 12 hours, to determine the diagnostic value of the CRP in predicting bacterial infection. This study concluded that a CRP value >40mg/L detected 79% of bacterial infections with 90% specificity; this data included patients with bacterial pneumonia and 'wheezy bronchitis'. Bacterial pneumonia was diagnosed in patients with lobar pneumonia and a high fever who demonstrated a response to treatment (normalisation of temperature) within 12 - 24 hours. Importantly, the authors used the CRP value to guide their decision not to prescribe antibiotics in 88% of children

with probable viral infections. However, the 16 cases of lobar pneumonia were all given antibiotics, irrespective of the CRP value, as all were presumed to be of bacterial origin. Only 6 of the 16 patients had either a positive blood culture (one patient) or positive bacterial antigen tests (five patients), highlighting the difficulty of establishing a definitive aetiological diagnosis in cases of pneumonia. The CRP values of the patients with lobar pneumonia were not independently reported.[26]

Babu *et al.* evaluated the usefulness of CRP measurements in children with acute lower respiratory tract infections. 65 children were classified into three groups: - pneumonia, bronchitis or normal. This classification was based on radiological findings (not specified in the report). The mean CRP values were 157mg/L for patients with pneumonia, 7.8mg/L for patients with bronchiolitis/ acute bronchitis and 3.5mg/L for normal children. All patients with pneumonia had a CRP value greater than 83mg/L and none of the patients with acute bronchitis and bronchiolitis had a CRP level greater than 35mg/L. The authors concluded that the CRP determination might be useful in the diagnosis and assessment of children with pneumonia.[27]

Some studies have been less enthusiastic about the ability of CRP determinations to distinguish bacterial from viral pneumonia. In a study from Oxford, England, 57 patients admitted with pneumonia were extensively investigated in an attempt to identify an aetiological agent. Tests included bacterial cultures and viral isolation techniques, antigen testing and serological tests for a wide variety of pathogens causing pneumonia, and chest radiographs. CRP levels [mean (SD)] could not distinguish between patients with bacterial pneumonia [12.9(12.9) mg/dl], viral pneumonia [9.4(12.4) mg/dl] or aetiologically undiagnosed pneumonia [11.8(10.7) mg/dl].[13]

In a prospective, population based study in Kuopio, Finland, 201 cases of radiologically confirmed community acquired pneumonia were identified. CRP

levels were unable to distinguish between pneumococcal infection, mycoplasmal and/or chlamydial infection, and viral pneumonia. Mean CRP concentrations (95% confidence intervals) were 26.8 (20.1 - 33.5) mg/L in the pneumococcal group; 31.8(20.5 - 33.1) mg/L in the mycoplasmal/ chlamydial infections; and 26.1(19.1 - 33.1) mg/L. in viral infections.[28]

Complicating the picture is the fact that several viral pathogens that cause pneumonia are also associated with high CRP levels. Adenovirus, influenza and parainfluenza viruses, and respiratory syncytial virus can cause CRP elevations that are seen in bacterial infections.[13, 29]

Thus, the data regarding the usefulness of CRP determinations in children with pneumonia yield conflicting information. The difference in mean CRP values in bacterial and viral pneumonia is generally statistically significant; whether this difference influences clinical practice regarding the initiation of antibiotic therapy is unknown. No definite recommendations have been made with regard to the initiation of antibiotic therapy in children with pneumonia.

In Soweto, Johannesburg, pneumonia is the commonest admission diagnosis and accounts for 36.9% of all paediatric admissions to the Chris Hani-Baragwanath Hospital (CHBH). [30] Tests that are commonly performed to diagnose bacterial pneumonia include blood cultures, total white cell count (WCC), CRP and chest radiographs. Although blood cultures have a poor sensitivity, they provide valuable information about common aetiological agents and antibiotic sensitivities when positive.

Large proportions (30%) of all children admitted to the CHBH are co-infected with HIV. 85.4% of all HIV infected children admitted to the CHBH are diagnosed with pneumonia, compared with 50.9% of HIV uninfected children.[31] The immunosuppression caused by HIV infection may influence CRP responses in

bacterial infection. The effect of immunosuppression on the CRP response has been evaluated in leukaemic children.[32] This study showed that chemotherapy-induced immunosuppression had no effects on the CRP responses in children with proven bacterial and fungal septicaemia. However, there is no data of which the author is aware, that has investigated the influence of HIV infection on CRP levels in children with pneumonia.

CRP levels are routinely measured in children diagnosed with pneumonia at the CHBH; and it is used as a screening test for bacterial infection. As a screening test, it should have a high sensitivity, as no case of bacterial pneumonia should go undetected. The CRP measurements should also have a good specificity and negative predictive value, especially important in the resource-limited settings of the developing world.

The cost of CRP testing should be justified in resource-limited settings and costs analyses regarding the use of CRP measurements in children with pneumonia are lacking. Babu *et al.* conducted their study in Chandigarh, India - in a clinical setting typical of the developing world and recommended that the CRP, an 'inexpensive' test, is a valuable tool for the rapid diagnosis of pneumonia. However, no costs analyses were reported in their study.[27]

1.3 Objectives of the study

The purposes of this study are to evaluate whether the initial CRP measurement allows discrimination between children with bacterial and viral pneumonia. Secondly, the effect of HIV infection on CRP responses in children with bacterial pneumonia will be assessed. Finally, a costs analysis will be performed to investigate the value of routine CRP measurements (used as a screening test) in children with pneumonia, in a clinical setting of a developing country.

2.0 MATERIALS AND METHODS

2.1 Study Design

A retrospective review of records of children diagnosed with pneumonia.

2.2 Study Population

The Pneumococcal Unit of the South African Institute of Medical Research (SAIMR), based at the Chris Hani-Baragwanath Hospital (CHBH), Soweto, South Africa has been investigating the aetiological agents causing pneumonia at this institution. Patients enrolled have had the following investigations performed at the time of admission: a full blood count and differential (FBC), CRP, chest radiograph, blood culture, immunoflorescent monoclonal antibody tests for respiratory viral pathogens in nasopharyngeal aspirates and serological tests for HIV. The immunoflorescent test screened for the following viruses: respiratory syncytial virus, adenovirus, influenza A and B and parainfluenza 1 and 3. Records of patients admitted from March 1997 to August 1998 were analysed.

The following inclusion criteria was used to select patients:

1. Infants and children aged between 6 weeks and 5 years
2. Diagnosis of pneumonia based on the presence of the following criteria:
    a) An infiltrate on the chest radiograph [alveolar consolidation (either 'lobar' pneumonia or 'bronchopneumonia') or interstitial pneumonia as defined by Friis[17]]

    and

    b) respiratory rate >50 breaths per minute in infants and a respiratory rate >40 breaths per minute in children over 1 year of age

    and / or:

    c) lower chest wall indrawing (or intercostal recessions in malnourished children)
3. Onset of symptoms within two weeks prior to admission

The following exclusion criteria were applied:

1. Re-admission within two weeks of being discharged from any hospital
2. Antibiotic therapy in the week preceding admission except for co-trimoxazole prophylaxis in infants born to HIV-infected mothers
3. A diagnosis of tuberculosis or asthma
4. The presence of other significant foci of infection (for example, intracranial infections, septic arthritis, osteomyelitis, infective endocarditis, cellulitis, lung abscess or other tissue abscesses)
5. Serious co-existing illnesses involving major organ systems (for example, congenital heart disease, chronic renal insufficiency)
6. A lack of the following results:
    a) CRP value
    b) Final blood culture and viral immunoflorescent monoclonal antibody results
    c) HIV serology

All study patients with pneumonia were exclusively allocated to one of the following groups:

1. Group 1: bacterial pneumonia (positive blood culture)
2. Group 2: viral pneumonia (positive nasopharyngeal immunoflorescent monoclonal antibody assay)
3. Group 3: pneumonia of mixed aetiology (positive blood culture and nasopharyngeal immunoflorescence)
4. Group 4: pneumonia of unknown aetiology (sterile blood cultures and non-reactive nasopharyngeal immunoflorescent monoclonal antibody assays)

2.3 Measurements

CRP values were determined by immunoturbidometry (Boehringer Mannheim Hitachi 717 Automated Analyzer). CRP values were reported in mg/L. CRP results were catergorised into groups based on increments of 10mg/L. This catergorisation was necessary since the laboratory reported some CRP values of 3mg/L or less as " less than or equal to 3mg/L (≤ 3mg/L)" and some CRP values of 200mg/L or more as "greater than or equal to 200mg/L (≥ 200mg/L)". Certain CRP values below 3mg/L or above 200mg/L were accurately quantified. The total WCC was calculated using an automated full blood analyzer (Coulter STKR). HIV testing was done by the ELISA method (Abbott Laboratories 3rd generation tests) and polymerase chain reaction (Roche Laboratories) where appropriate. Bacterial cultures were performed on blood agar, chocolate agar and Maconkey agar. Nasopharyngeal aspirates were submitted for immunoflourescence and shell vial culture centrifugation techniques.

2.4 Statistical Analysis

The median CRP values with the 25% to 75% interquartile range (IQR) were determined for each category of patients and this data is represented by box and whisker plots. Mean CRP values were not reliable, as the CRP values at the extremes of the range were not always precisely quantified (see above). Those CRP values reported as "≥200mg/L" by the laboratory were recorded as 200mg/L for the purposes of statistical analysis. However some CRP values greater than 200mg/L were accurately quantified by the laboratory. Where available, accurately quantified CRP values were used in the statistical analysis.

The CRP values in the various aetiological groups (Groups 1-4) were compared for statistical significance by the Median test. The Median test is a non-parametric test and is described as a 'crude' version of the Kruskal-Wallis ANOVA test. The Median

test determines the median CRP value for all the patients (disregarding the aetiological group), the so-called 'common median'. The number of cases in each aetiological group (Groups 1-4) that fall above or below the 'common median' are entered into a 2×4 contingency table and the *Chi-squared* value is computed. Under the null hypothesis (all samples come from populations with identical medians), approximately 50% of all CRP values in each aetiological group are expected to fall above (or below) the common median. The Median test is particularly useful when the variable contains artificial limits, and many cases fall at either extreme of the scale.[33] In this analysis, the CRP values at either extreme were frequently reported as ≤ 3mg/L or ≥ 200mg/L. In this case, the Median test is in fact the most appropriate method for comparing groups.

Various threshold CRP values (beginning at 10 mg/L and increasing in increments of 10 mg/L to a value of 200 mg/L) were selected and tested for their ability to differentiate bacterial from viral pneumonia. A 2 × 2 table was constructed and bacterial (Group 1) and viral pneumonia (Group 2) were compared to each other, using the designated threshold CRP value. Statistical significance was assessed by a one-tailed Fischer's exact test. A *P* value of less than or equal to 0.05 was considered significant.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of various CRP levels predicting bacterial pneumonia (groups 1 and 3) were calculated as defined by Feinstein.[34]

2.4.1 Receiver-operating characteristic (ROC) curves

ROC curves were created to determine whether a particular CRP value predicted a positive blood culture indicating bacterial infection. These curves were constructed individually for HIV-infected and -uninfected patients as well as for the total number of patients. ROC curves are created utilising the sensitivity and specificity of a

particular CRP cut-off value. The sensitivity and specificity values are calculated as percentages. The sensitivity (y-axis) of a CRP value is plotted against its specificity that has been subtracted from 1(or 100%). The latter is represented as "1-specificity" on the x-axis. The individual plots are joined linearly to complete the ROC curve.[35]

Receiver-operating characteristic (ROC) curves are constructed to permit selection of threshold CRP values. Ideally a perfect diagnostic test would give neither false-positive nor false-negative results. The optimal cut-off limit for an abnormal test result is one that produces a point nearest the upper left corner on the ROC graph.[36] It is preferable to treat several children with false-positive diagnoses rather than miss a single child with bacterial pneumonia, so ROC curves were used to identify threshold values with maximum sensitivities.

2.5 Ethical considerations

Ethical clearance for this study has been obtained from the Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand, Johannesburg (Clearance certificate number M980906).

3.0 RESULTS

One thousand and nine hundred completed records were available for review from which 570 records were selected for analysis. These patients were catergorised into four groups, based on the aetiology of the pneumonia. 55 patients were catergorised into Group 1 - bacterial pneumonia, 145 into Group 2 - viral pneumonia, 11 into Group 3 - mixed pneumonia and the majority, 359 patients, were catergorised into Group 4 - pneumonia of unknown aetiology.

1330 patients were excluded from the analysis (Table 1). The majority of these patients had either received antibiotic therapy prior to admission or did not have nasopharyngeal aspirates submitted for viral studies.

Antibiotic therapy, usually oral amoxycillin or intramuscular ampicillin was administered to some children who presented to the community health clinics with severe pneumonia. These patients were thus referred to CHBH after receiving antibiotic therapy, in accordance with the standard practice in the region. Nasopharyngeal aspirates (for viral studies) were not collected from patients admitted over weekends due to staff shortages at CHBH. Patients with a diagnosis of tuberculosis or other underlying disorders were excluded as these conditions may independently influence CRP levels. Asthmatics, who often present with similar clinical findings as patients with pneumonia, were excluded. Some specimens submitted for CRP, blood culture and/or HIV testing were not reported for various reasons: specimens were either not taken or lost and some tests were not performed due to laboratory accidents occurring. HIV testing was performed at the discretion of the admitting doctor, usually if clinical features suggestive of HIV infection are present in the patient. In addition, parental consent is required for HIV testing. Children admitted to a health facility in the previous two weeks were excluded because of the possibility of having acquired nosocomial pneumonia.

Table 1. Summary of patients excluded from analysis (n=1330).

Reason for exclusion	Number of patients
Antibiotic therapy prior to admission	522
Nasopharyngeal (viral) immunoflourescent antibody tests not done	388
No HIV result available	99
Other illnesses, including asthma	78
Tuberculosis diagnosed	55
No CRP result	68
No blood culture result	35
Inadequate nasopharyngeal specimens	32
Admission to a health facility in the prior 2 weeks	30
Other (2 or more of the above reasons)	23

3.1 Demographic Data

The age, sex and nutritional status (as defined according to the Wellcome classification[37]) of patients were recorded in 514 (90.2%) patient records. In this analysis, pneumonia was commoner in male patients (M: F = 1.37:1). Viral pneumonia occurred at a younger age than bacterial pneumonia. The average age of patients diagnosed with viral pneumonia was 9.4 months compared to the average age of 15.7 months for patients with bacterial pneumonia. 42.8% of the 570 patients were co-infected with HIV. The demographic data are summarised in Tables 2a-d.

A significant proportion (32.6%) of children admitted with pneumonia were malnourished (underweight for age, marasmus, kwashiorkor or marasmic-kwashiorkor). 10% of all children were severely malnourished (marasmus, kwashiorkor or marasmic-kwashiorkor). With regard to severe malnutrition, marasmus occurred more commonly than oedematous malnutrition (kwashiorkor, marasmic-kwashiorkor) in HIV infected children compared to HIV uninfected children ($P = 0.001$).

Over half (56.6%) of the children with bacterial pneumonia were malnourished (Table 2a). Although malnutrition occurred more commonly in HIV infected children, there was no significant difference in the nutritional status (well nourished versus malnourished) of HIV infected and uninfected children with bacterial pneumonia ($P = 0.097$)

Table 2a. Demographic data of patients in Group 1 (Bacterial pneumonia)

	Number	Male (%)	Female (%)	Average age [range] in months	NUTRITIONAL STATUS				
					Normal (%)	Under-weight (%)	Kwashiorkor (%)	Marasmus-(%)	Marasmic-Kwashiorkor (%)
TOTAL	53	31 (58.5)	22 (41.5)	15.7 [2-60]	23 (43.4)	25 (47.2)	3 (5.7)	2 (3.8)	0
HIV+	34	19 (55.9)	15 (44.1)	16.5 [2-60]	12 (35.3)	21 (61.8)	0	1 (2.9)	0
HIV-	19	12 (63.2)	7 (36.8)	13.3 [2-54]	11 (57.9)	4 (21.1)	3 (15.8)	1 (5.3)	0

The majority (82.0%) of children with viral pneumonia were well nourished (Table 2b) and no significant difference was noted in the nutritional status of HIV infected and uninfected children ($P = 0.068$).

Table 2b. Demographic data of patients in Group 2 (Viral pneumonia)

	Number	Male (%)	Female (%)	Average age [range] in months	NUTRITIONAL STATUS				
					Normal (%)	Under-weight (%)	Kwashiorkor (%)	Marasmus-(%)	Marasmic-Kwashiorkor (%)
TOTAL	133	80 (60.2)	53 (39.8)	9.4 [2-47]	109 (82.0)	18 (13.5)	2 (1.5)	3 (2.3)	1 (0.8)
HIV+	22	14 (63.6)	8 (36.4)	10.8 [2-42]	15 (68.2)	5 (22.7)	0	1 (4.5)	1 (4.5)
HIV-	111	66 (59.5)	45 (40.5)	9.1 [2-47]	94 (84.7)	13 (11.7)	2 (1.8)	2 (1.8)	0

Most of the children (70%) with mixed pneumonia were well nourished and there was no significant difference in the nutritional status of HIV infected and uninfected children ($P = 0.67$). However, the small number of cases (n=10) in the group of mixed pneumonia limits the interpretation of the data regarding nutritional status (Table 2c).

Table 2c. Demographic data of patients in Group 3 (*Mixed pneumonia*)

	Number	Male (%)	Female (%)	Average age [range] in months	NUTRITIONAL STATUS				
					Normal (%)	Under-weight (%)	Kwashiorkor (%)	Marasmus-(%)	Marasmic-Kwashiorkor (%)
TOTAL	10	4 (40)	6 (60)	7.5 [3-19]	7 (70)	3 (30)	0	0	0
HIV+	4	1 (25)	3 (75)	10.3 [4-19]	3 (75)	1 (25)	0	0	0
HIV-	6	3 (50)	3 (50)	5.7 [3-14]	4 (66.7)	2 (33.3)	0	0	0

Children with pneumonia of unknown aetiology were mostly well nourished (59.4%) (Table 2d). However, in this group significantly more HIV infected children were malnourished when compared to HIV uninfected children ($P< 0.001$).

Table 2d. Demographic data of patients in Group 4 (Pneumonia of unknown aetiology)

	Number	Male (%)	Female (%)	Average age [range] in months	NUTRITIONAL STATUS				
					Normal (%)	Under-weight (%)	Kwashiorkor (%)	Marasmus- (%)	Marasmic-Kwashiorkor (%)
TOTAL	318	182 (57.2)	136 (42.8)	11.6 [2-60]	189 (59.4)	83 (26.1)	10 (3.1)	30 (9.4)	6 (1.9)
HIV+	155	81 (52.3)	74 (47.7)	11.9 [2-60]	75 (48.4)	52 (33.5)	3 (1.9)	23 (14.8)	2 (1.3)
HIV -	163	101 (62.0)	62 (38.0)	11.4 [2-48]	114 (70.0)	31 (19.0)	7 (4.3)	7 (4.3)	4 (2.5)

3.2 Blood Cultures

Recognised bacterial pathogens causing pneumonia accounted for 66 of the 168 (39.29%) positive blood cultures. These organisms are listed in Table 3. The majority of significant blood cultures were attributable to *Streptococcus pneumoniae* (52.3%), *Staphylococcus aureus* (15.0%) and *Haemophilus influenzae* (13.4%). Pneumonia caused by *Salmonella* species was regarded as a significant infection in HIV infected children.[38] However *Salmonella* species was considered a contaminant in HIV uninfected children. One patient was co-infected with *S. pneumoniae* and *H. influenzae*.

Table 3. Significant blood cultures

Organism	GROUP 1 Bacterial pneumonia No. of patients (%)			GROUP 3 Mixed pneumonia No. of patients (%)		
	HIV +	HIV -	Total	HIV +	HIV -	Total
Streptococcus pneumoniae	23 (34.3)	8 (12.0)	31 (46.3)	2 (3.0)	2 (3.0)	4 (6.0)
Haemophilus influenzae	6 (9.0)	3 (4.5)	9 (13.4)	0	0	0
Staphylococcus aureus	4 (6.0)	2 (3.0)	6 (9.0)	1 (1.5)	3 (4.5)	4 (6.0)
Streptococcus viridans	2 (3.0)	3 (4.5)	5 (7.5)	1 (1.5)	0	1 (1.5)
Streptococcus agalactiae	0	1 (1.5)	1 (1.5)	0	0	0
Escherichia coli	2 (3.0)	0	2 (3.0)	0	1 (1.5)	1 (1.5)
Klebsiella pneumoniae	0	1 (1.5)	1 (1.5)	0	0	0
Pseudomonas	0	1 (1.5)	1 (1.5)	0	0	0
Salmonella species	0	0	0	1 (1.5)	0	1 (1.5)

102 of 168 (60.71%) positive blood cultures yielded organisms that were most likely contaminants and not true pathogens. These organisms are listed in Table 4. The vast majority of the contaminants were due to *Staphylococcus epidermidis*. Patients with contaminant blood cultures were allocated to either Group 2 or 4 (depending on the viral studies).

Table 4. List of organisms causing contaminant blood cultures

Organism	Total (%)	Group 2 - Viral pneumonia No. of patients (%)	Group 4 - Unknown aetiology pneumonia No. of patients (%)
Staphylococcus epidermidis	80 (78.4)	26 (25.5)	54 (52.9)
Commensal Corynebacteria	10 (9.8)	2 (2.0)	8 (7.8)
Bacillus species	3 (2.9)	1 (1.0)	2 (2.0)
Propionibacterium acnes	3 (2.9)	2 (2.0)	1 (1.0)
Streptococcus pyogenes	2 (2.0)	0	2 (2.0)
Enterococcus faecium	1 (1.0)	0	1 (1.0)
Pasteurella	1 (1.0)	0	1 (1.0)
Salmonella species	1 (1.0)	1 (1.0)	0
Other	1 (1.0)	0	1 (1.0)

3.2.1 **Median CRP values of the significant bacterial pathogens**

The median CRP values were determined for each bacterial pathogen (Table 5). With the exception of *S.viridans*, bacterial pneumonia caused by *S.pneumoniae*, *H.influenzae*, *S.aureus* and *E.coli* were associated with median CRP values greater than 50mg/L. The low CRP values associated with *S.viridans* will be discussed later (Section 3.5). Pneumonia caused by *S.pneumoniae* was associated with higher CRP values compared to the other bacterial pathogens. Bacterial pneumonia caused by *S.agalactiae*, *K.pneumoniae*, *Pseudomonas* and *Salmonella* species were each documented in a single case, therefore the median CRP value could not be determined for these infections. The single patient co-infected with *S.pneumoniae* and *H.influenzae* was excluded from this particular analysis.

Table 5. CRP values according to bacterial pathogen

	Number of patients	Median CRP	Minimum CRP	Maximum CRP	Lower (25%) quartile	Upper (75%) quartile
Streptococcus pneumoniae	34	200	12	402	160	250
Haemophilus influenzae	8	110.5	5	264	43.5	153
Staphylococcus aureus	10	54	6	657	17	106
Streptococcus viridans	6	12.5	3	56	6	20
Escherichia coli	3	127	28	200	-	-

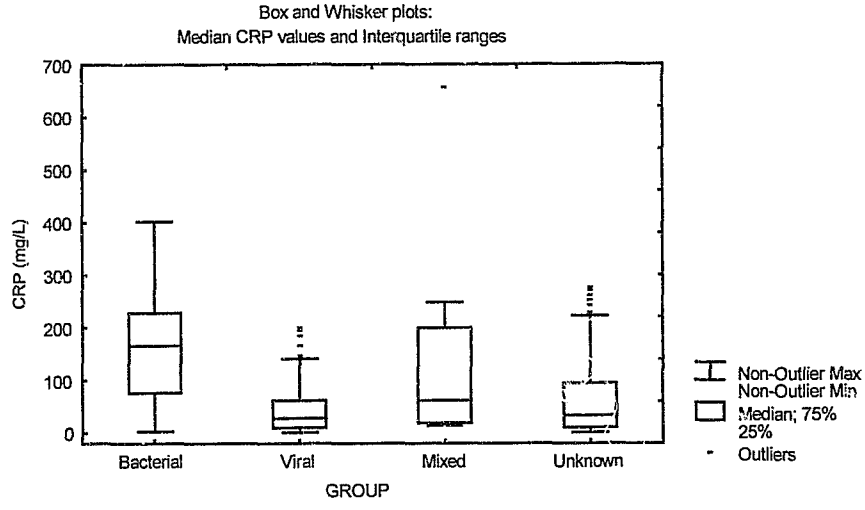
3.3 Median CRP values

All 570 patients were allocated to the four groups described earlier. The median CRP values and 25% - 75% interquartile ranges were determined for each aetiological group and are shown in Table 6a. Figure 1 represents the median CRP value and interquartile range for each aetiological group as a box and whisker plot.

Table 6a. Median CRP values (All patients)

Group	Number	Median	Interquartile range (25% - 75%)
Group 1 Bacterial pneumonia	55	160	77 - 230
Group 2 Viral pneumonia	145	24	7 - 62
Group 3 Mixed pneumonia	11	56	17 - 200
Group 4 Unknown aetiology	359	27	8 - 90

Figure 1. Median CRP values and interquartile ranges according to aetiological group



The median CRP value in each aetiological group was separately calculated for HIV infected and uninfected patients (Tables 6b- c). HIV infection did not alter the CRP responses in patients with pneumonia, irrespective of the aetiology of the pneumonia. In patients with bacterial pneumonia, the median CRP was higher in HIV infected children (195mg/L) compared to HIV uninfected children (113mg/L). However, there were no significant differences in the CRP responses between HIV infected and HIV uninfected children, regardless of the aetiology of the pneumonia (Table 6d)

Table 6b. Median CRP values (HIV positive patients)

Group	Number	Median	Interquartile range (25% - 75%)
Group 1 Bacterial pneumonia	36	195	99.5 - 234.5
Group 2 Viral pneumonia	25	30	5 - 90
Group 3 Mixed pneumonia	5	84	56 - 90
Group 4 Unknown aetiology	178	27	7 - 126

Table 6c. Median CRP values (HIV negative patients)

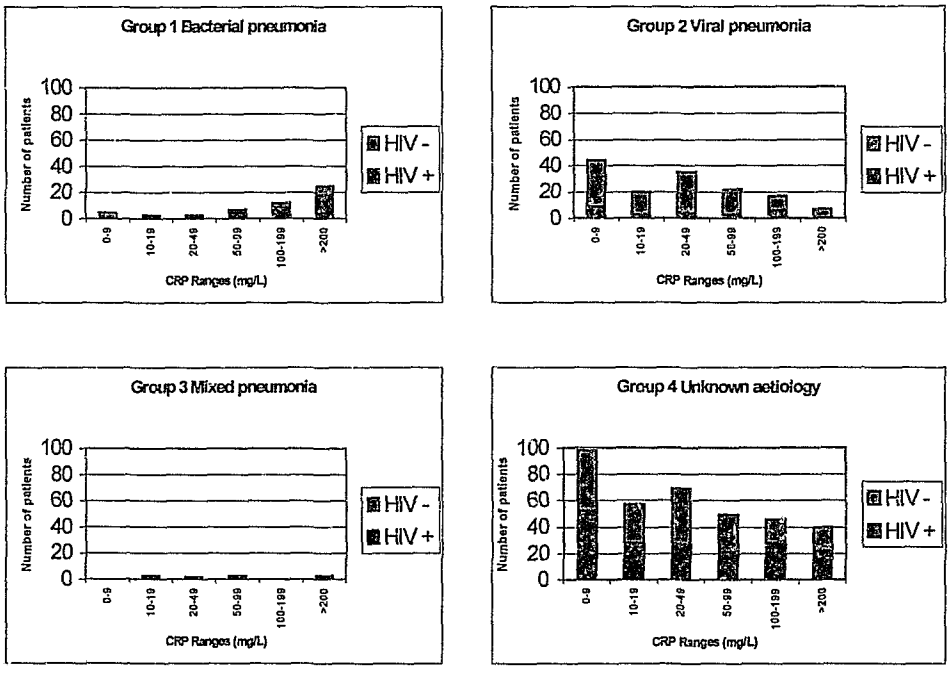
Group	Number	Median	Interquartile range (25% - 75%)
Group 1 Bacterial pneumonia	19	113	6 - 230
Group 2 Viral pneumonia	120	23.5	8 - 60.5
Group 3 Mixed pneumonia	6	22.5	14 - 200
Group 4 Unknown aetiology	182	27.5	9 - 73

Table 6d. Comparison of median CRP values in HIV infected and uninfected children.

	Median CRP		P value
	HIV +	HIV -	(Median test)
Bacterial pneumonia	195	113	0.19
Viral pneumonia	30	23.5	0.74
Mixed pneumonia	84	22.5	0.38
Pneumonia of unknown aetiology	27	27.5	0.87

The bar graphs in Figure 2 show the distribution of the CRP ranges according to the aetiological groups and HIV status. The distribution of the CRP ranges is skewed in each group. Higher values (CRP > 50mg/L) predominated in bacterial pneumonia (Group 1) whilst lower values (CRP < 50mg/L) predominated in viral pneumonia (Group 2) as well as in pneumonia of unknown aetiology (Group 4). Appendix 1 shows the detail of the total number of patients distributed according to CRP range, aetiological group and HIV status.

Figure 2. Distribution of CRP ranges



3.3.1 Comparison of CRP values in the various aetiological groups

The Median test was used to compare the median CRP values of the various aetiological groups (Groups 1-4) (see Methods and Materials for details). The computation is shown in Table 7.

Table 7. Comparison of CRP values according to aetiology - Median test

	Median Test, Common Median = 30.0 Independent variable : Aetiological Group Chi-Square = 23.548, degrees of freedom = 3, $P < 0.0001$				
Dependent variable: CRP	Group 1: Bacterial pneumonia	Group 2: Viral pneumonia	Group 3: Mixed pneumonia	Group 4: Pneumonia of unknown aetiology	Total
≤ Median: Observed (O)	11.00	82.00	5.00	190.00	288.0
Expected (E)	27.79	73.26	5.56	181.39	
(O) - (E)	- 16.79	8.74	-0.56	8.61	
> Median: Observed (O)	44.00	63.00	6.00	169.00	282.0
Expected (E)	27.21	71.74	5.44	177.61	
(O) - (E)	16.79	- 8.74	0.56	- 8.61	
Total: Observed	55.0	145.0	11.0	359.0	570.0

The greatest proportion of patients with CRP values above the common median is in the group with bacterial pneumonia (Group 1). The greatest proportion of patients with CRP values below the common median is in the group with viral pneumonia (Group 2). Thus, the Median test shows that children with bacterial pneumonia have significantly higher median CRP values than children with either viral or aetiologically undiagnosed pneumonia ($P < 0.001$).

A log-linear analysis was performed to compare the CRP values in all of the aetiological groups. This analysis revealed that patients with bacterial pneumonia had significantly higher CRP values compared to the common median whilst

patients with either viral or pneumonia of unknown aetiology had CRP values that were significantly lower than the common median.

3.3.2 Comparison of CRP values in bacterial and viral pneumonia

2 × 2 tables were constructed to assess the value of using different threshold CRP values for diagnosing bacterial (Group 1) versus viral (Group 2) pneumonia. The threshold CRP values that were chosen began at a value of 10mg/L and increased in increments of 10 to a maximum value of 100mg/L. Statistical significance was assessed by means of a one-tailed Fishers exact test. This analysis was performed for the total group of patients and individually for the HIV-infected and HIV-uninfected children. An example is shown below where a threshold CRP value of 10mg/L is used to differentiate bacterial from viral pneumonia in all patients:

	Bacterial pneumonia	Viral pneumonia
CRP ≥ 10mg/L	50	101
CRP < 10mg/L	5	44

$P = 0.0009$

The calculated *P* value in the above example is 0.0009. Table 8 summarises these calculations.

Table 8. Tabulation of *P* values for threshold CRP values used to differentiate bacterial from viral pneumonia.

	P v[illegible]		
Threshold CRP value (mg/L)	HIV positive	HIV negative	Total
10	0.0001	0.5185	0.0009
20	0.0006	0.1240	< 0.0001
30	0.0017	0.0423	< 0.0001
40	0.0001	0.0195	< 0.0001
50	< 0.0001	0.0075	< 0.0001
60	< 0.0001	0.0019	< 0.0001
70	< 0.0001	< 0.0001	< 0.0001
80	< 0.0001	0.0031	< 0.0001
90	0.0001	0.0009	< 0.0001
100	0.0001	0.0007	< 0.0001

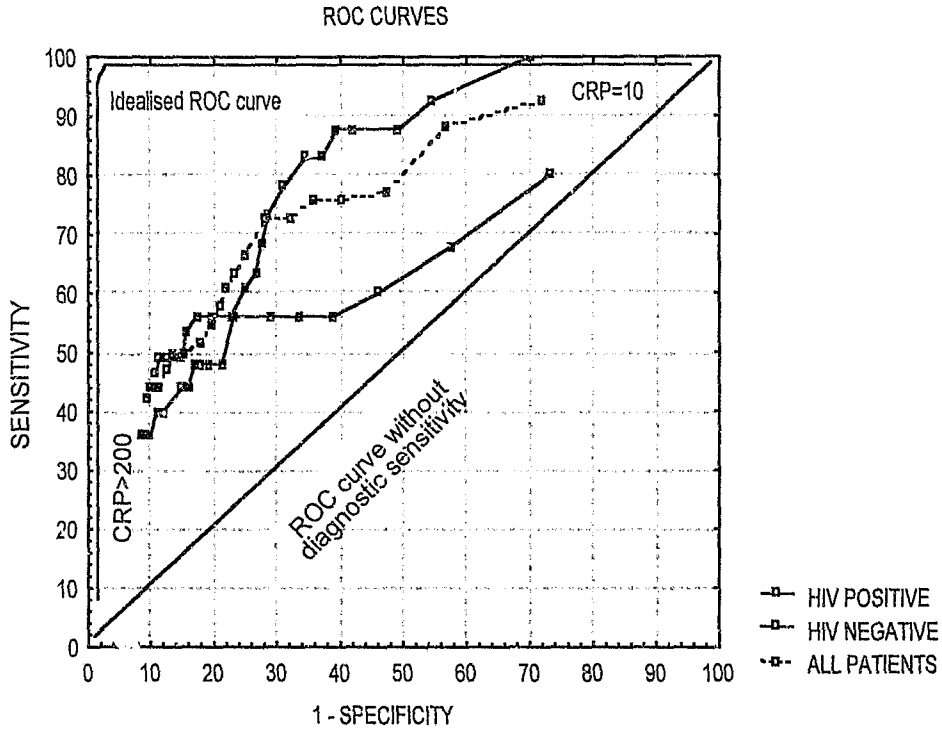
Threshold CRP values of 10mg/L and 20mg/L were unable to distinguish between bacterial and viral pneumonia in HIV uninfected patients. Thresh old CRP values of ≥30mg/L were statistically useful in distinguishing between bacterial and viral infection in HIV uninfected patients. In patients who are co-infected with HIV or in whom the HIV status is unknown, all the chosen threshold CRP values were statistically useful in separating bacterial from viral pneumonia.

3.4 ROC curve plots

The sensitivity, specificity, positive predictive value and negative predictive value of various threshold CRP values that predicted a positive blood culture were calculated. These results are shown in Appendices 2 – 4. These analyses were independently performed in HIV infected and uninfected children, as well as in all patients with pneumonia. Generally, threshold CRP values at the lower range (≥10mg/L) were associated with good sensitivities (80-100%). The specificities associated with threshold CRP values commonly used in clinical practice, i.e. ≤ 40mg/L, were poor (26.9-53.8%). The CRP threshold values showed better sensitivities in HIV positive children. The negative predictive value was close to or exceeded 90% in most cases, whilst the positive predictive values correlated poorly with the threshold CRP values.

The ROC curves (Figure 3) were constructed by plotting the sensitivity (in %) of a particular threshold CRP value against that value's specificity (in %) subtracted from 100%. In each ROC curve plot, the point on the extreme right-hand side represents a CRP value of 10mg/L. The point immediately to the left of the above-mentioned one represents a CRP value of 20mg/L. In this order, each point to the immediate left of the previous one represents an increment in the CRP value of 10 units (in mg/L). The last point on the plot is located on the extreme left-hand side of the plot and represents a threshold CRP value of ≥ 200mg/L.

Figure 3. Receiver operating characteristic curve constructed for CRP values that predict bacterial pneumonia.



The ROC curve plots do not reveal any threshold CRP value that can reliably predict proven bacterial pneumonia. Interestingly, the best ROC curve plots are shown in HIV positive patients, where a threshold CRP value of 10mg/L predicted all cases of bacterial pneumonia. The ROC curve plots show that no particular threshold CRP value can be chosen to predict bacterial pneumonia in HIV uninfected patients. For every five children with bacterial pneumonia, one case would be missed if a threshold CRP value of 10mg/L was used in HIV uninfected children. Approximately one child in every ten children with bacterial pneumonia will be missed if a threshold CRP value of 10mg/L (associated with the best sensitivity) is used for children whose HIV status is unknown (Table 9).

Table 9. ROC curve plot data associated with a threshold CRP value of 10mg/L

	Sensitivity	Specificity	1- specificity	PPV	NPV
HIV +	100	30.0	70.0	22.4	100
HIV -	80	26.9	73.1	9.1	94.2
All patients	92.4	28.2	71.8	14.4	96.6

PPV = positive predictive value, NPV = negative predictive value

3.5 *Streptococcal viridans* pneumonia

Debate exists as to whether or not *Streptococcus viridans* causes community acquired pneumonia. Although *S.viridans* is thought to be a blood culture contaminant in about 50% of cases,[39] it has been implicated as a cause of community acquired pneumonia in South African adults.[40] All analyses performed thus far in this report have regarded *S.viridans* as a significant pathogen. The data was re-analyzed disregarding *S.viridans* as a significant pathogen and instead considering it as a blood culture contaminant. Six patients (3 HIV positive) cultured *S.viridans* on blood agar. Five of these patients, with CRP values ranging from 3 to 20mg/L were re-allocated to Group 4; one patient, with a CRP value of 56mg/L was re-allocated to Group 2 on the basis of positive immunoflorescent tests for adenovirus and parainfluenza. These CRP ranges are shown in Appendix 5, and the data used for the ROC curve plot are shown in Appendices 6-8.

The exclusion of *Streptococcus viridans* from the group of bacterial pneumonia increased the median CRP value from 160mg/L to 195mg/L. However the increase in the median CRP value was not significant (P=0.63, Median test). The median CRP values in the viral and mixed pneumonia groups remained unchanged (see Table 6a). The median CRP value in the aetiologically undiagnosed group decreased slightly to 25mg/L (as compared to 27mg/L when *S.viridans* was regarded as a significant pathogen).

The CRP values in the bacterial and viral pneumonia groups were compared (as described in section 3.3.2). This analysis revealed a statistical difference ($P < 0.05$) when threshold CRP values, ranging from 10mg/L to 100mg/L, were used to differentiate bacterial from viral pneumonia in all patients (Table 10). A threshold CRP value of 10mg/L could not differentiate bacterial from viral pneumonia in HIV uninfected patients, the only subgroup of patients where the CRP level was not significantly different in bacterial and viral pneumonia. These results are very similar to the previous analyses that regarded *S.viridans* as a significant pathogen. Therefore, the exclusion of *Streptococcus viridans* as a bacterial pathogen did not significantly influence the results when threshold CRP values were used to differentiate bacterial and viral pneumonia.

Table 10. Threshold CRP values comparing bacterial & viral pneumonia (*S.viridans* not significant)

	P value		
CRP Cut-off value (Mg/L)	HIV positive	HIV negative	Total
10	0.0002	0.1316	< 0.0001
20	0.0003	0.0143	< 0.0001
30	0.0003	0.0051	< 0.0001
40	< 0.0001	0.0027	< 0.0001
50	< 0.0001	0.0009	< 0.0001
60	< 0.0001	0.0002	< 0.0001
70	< 0.0001	0.0000	< 0.0001
80	< 0.0001	0.0006	< 0.0001
90	< 0.0001	0.0002	< 0.0001
100	< 0.0001	0.0001	< 0.0001

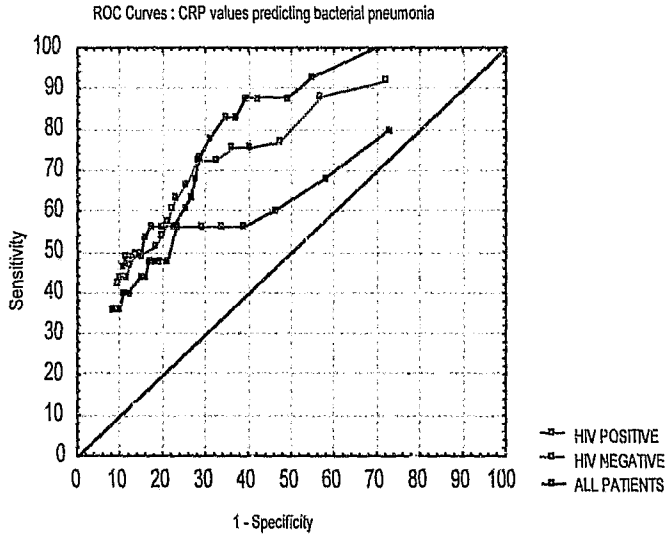
3.5.1 ROC curve plots (excluding *S.viridans*)

The sensitivity, specificity, positive - and negative - predictive values for CRP values that predicted bacterial pneumonia were calculated (Appendices 6-8). This data was used to construct the ROC curve plots (described in section 3.4). The ROC curve plots that regarded *S.viridans* as a contaminant were compared to ROC curve plots that regarded *S.viridans* as a significant organism (Figure 4).

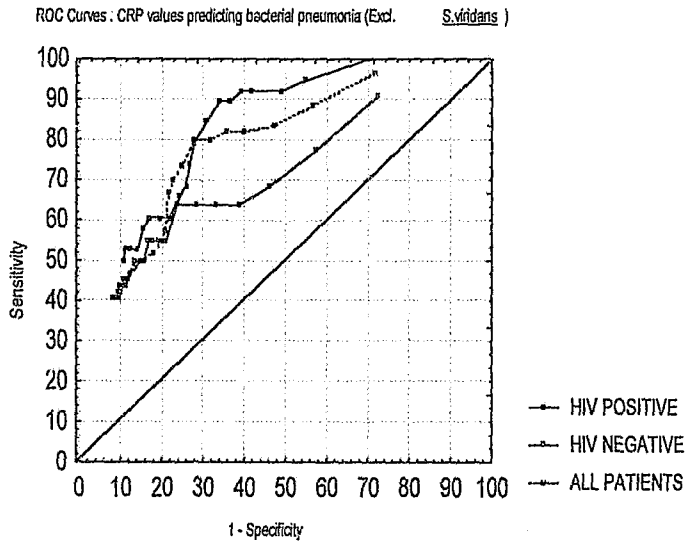
The ROC curve plot (Figure 4b), now created from the data that regarded *S.viridans* as a contaminant, showed an improvement in the sensitivity and diagnostic ability (as shown by a greater area under the curve) of the threshold CRP values that predicted bacterial pneumonia. Better ROC curve plots were constructed for HIV infected-children compared to HIV-uninfected children. The exclusion of *Streptococcus viridans* as a recognised bacterial pathogen resulted in an improvement in the sensitivity of the CRP test at a threshold value of 10mg/L. In HIV uninfected children, instead of missing 1 in 5 children with bacterial pneumonia, 1 in 10 children would now be missed.

Figure 4. Comparison of the ROC curve plots (a) including *S.viridans* in the group of bacterial pneumonia and (b) excluding *S.viridans* in the group of bacterial pneumonia.

(a)



(b)



3.6 Costs analysis

A costs analysis was performed to determine whether costs saved by sparing the use of antibiotics in children with viral pneumonia justified the costs of CRP testing. The cost of a single CRP determination at the SAIMR Laboratories in Johannesburg, South Africa is R14.18 (one US dollar is equivalent to six rand). A 7-day course of oral amoxycillin syrup costs R6.88. These costs are applicable to patients treated at government institutions; costs in the private sector are considerably more.

A costs analysis was determined in HIV infected children, choosing a threshold CRP value of 10mg/L (100% sensitivity for predicting bacterial pneumonia). The CRP measurements in the 244 HIV-infected children would cost R 3459.92. 61 children, who had CRP values of less than 10mg/L, would not have received antibiotics. The saving accrued by not administering antibiotics is R 419.68. If a threshold CRP value of ≥200mg/L is chosen (which has a high specificity and negative predictive value, minimising antibiotic usage) the savings accrued would be R 1396.6, still less than the cost of CRP testing.

In all patients with pneumonia, choosing a threshold CRP value of ≥200mg/L (with maximum specificity and negative predictive value) would result in R3405.60 being saved as a result of not using antibiotics. The cost of CRP testing in this group is R8082.60. Using a threshold CRP value of 10mg/L, R1011.36 would be saved by not prescribing oral amoxycillin for those patients with CRP values <10mg/L.

The costs analysis suggests that CRP testing may be more expensive as compared to the costs of antibiotic therapy in children with pneumonia. However, this costs analysis is a superficial one, and does not take into account the costs incurred in treating complications related to the use of antibiotics, for example antibiotic associated diarrhoea. Furthermore, the costs of not treating those children with bacterial pneumonia and a low CRP value, has not been taken into account.

4.0 DISCUSSION

The present study analysed the usefulness of the initial CRP measurement that is performed in children presenting with pneumonia. The CRP test is used to predict bacterial pneumonia, thus facilitating the appropriate use of antibiotics. In this study the CRP responses were studied in 570 children with pneumonia, the largest series reported so far. A considerable number (42.8%) of all patients in this study were also co-infected with HIV. The large number of patients in the present study has permitted only those patients with radiologically confirmed pneumonia and positive blood cultures to be classified as having bacterial pneumonia. Therefore the relationship between the CRP values in 55 children, a relatively large sample of children fulfilling strict criteria for bacterial pneumonia, could be reliably assessed.

The range of CRP values in the various aetiological groups showed distinct trends (Figure 2). The majority (80%) of children with bacterial pneumonia had CRP values >50mg/L whilst a majority of children with either viral (68.3%) or aetiologically undiagnosed (62.4%) pneumonia had CRP values <50mg/L (Appendix 1). The median CRP value was significantly higher (P <0.0001) in children with bacterial pneumonia compared to children with viral pneumonia or pneumonia of unknown aetiology (160 vs. 24 and 27mg/L). The distribution of the CRP values in the aetiologically undiagnosed group (Group 4) is interesting (Figure 2). Although there is a suggestion of a bimodal peak, it is not distinctly obvious. If CRP values could accurately distinguish bacterial from viral infection, a bimodal peak should be clearly evident. The CRP distribution in this group may suggest that most of the cases of blood culture negative-pneumonia are caused by viral infections. Mean CRP values and confidence intervals could not be determined for the various aetiological groups because of the method of reporting CRP results.

This study investigated the usefulness of using various threshold CRP values to distinguish between bacterial and viral pneumonia. In HIV uninfected patients, the lowest threshold CRP value that could statistically distinguish between bacterial and viral pneumonia is 30mg/L ($P = 0.04$). This threshold CRP value dropped to 10mg/L in HIV infected children ($P = 0.0001$). At CHBH, the HIV status of most children with pneumonia is not known to the clinician at the time of CRP testing. In children whose HIV status is unknown a threshold CRP value of 10mg/L could statistically distinguish between bacterial and viral pneumonia ($P = 0.0009$).

The construction of ROC curve plots permits the selection of an optimal threshold CRP value that predicts bacterial pneumonia. However, the threshold CRP value chosen to predict bacterial pneumonia should have a sensitivity of 100%, as no case of bacterial pneumonia should go untreated.

For HIV uninfected patients, ROC curve analysis (Figures 3 and 4) did not establish any appropriate threshold CRP value above which results could predict all cases of bacterial pneumonia. A threshold CRP value of 10mg/L was associated with a sensitivity of 80%, the maximum sensitivity in HIV uninfected patients. Thus if a CRP value of ≥10mg/L is used to predict bacterial pneumonia, one out of every five HIV uninfected patients would not be treated appropriately with antibiotics. In HIV infected patients, irrespective of whether or not *S.viridans* was considered as a significant pathogen, ROC curve analysis detected all cases of bacterial pneumonia if the CRP value is ≥ 10mg/L. The ROC curve plot constructed for HIV infected and uninfected children revealed that a threshold CRP value of 10mg/L predicted 92.4% of bacterial pneumonia. Thus if a threshold CRP value of 10mg/L were used to screen children from HIV endemic areas for bacterial pneumonia, approximately one in ten children with bacterial pneumonia would be missed.

The ROC curve plot constructed for HIV infected and uninfected patients demonstrates a poor specificity (28.17%) when correlated with the CRP value that predicts maximum sensitivity. This implies that many children with viral pneumonia will be treated inappropriately with antibiotics.

The present study is the first analysis that investigated the CRP response in HIV infected children with pneumonia. There was no significant difference in the CRP responses of HIV infected and uninfected children. In children with bacterial pneumonia, the median CRP value was higher in HIV infected children compared to HIV uninfected children (195mg/L versus 113mg/L), although this was not significant ($P = 0.19$). Interestingly, the ROC curve plots were better in HIV infected children compared to HIV uninfected children. In HIV infected children, a threshold CRP value of 10mg/L predicted all cases of proven bacterial pneumonia, although this value also included 64% of viral pneumonia.

The present study also determined the costs analysis of routine CRP testing in children with pneumonia. The cost of the test was compared to the cost of oral amoxycillin, the antibiotic routinely used as empiric therapy in the majority of patients. This analysis suggests that a routine CRP measurement performed in children presenting with pneumonia may not be appropriate in a resource limited environment. Costs analysis also suggests that the CRP test is more expensive than the savings gained by sparing the use of intravenous antibiotic therapy in a child with pneumonia. Intravenous fluids and antibiotics are administered to patients who are dehydrated and not tolerating oral fluids or feeds, or who are too ill to feed. In this case, intravenous ampicillin is prescribed, usually for 2 to 3 days. This will increase the total cost of antibiotics used (intravenous and oral) to R16.48 (assuming that 3 days of intravenous ampicillin is used). Using HIV infected children (who have the best ROC curve plots) as an example, a threshold CRP value of 10mg/L will result in 61 children being spared the use of antibiotics, saving

R1005.28. This saving is still considerably less than the cost of CRP testing in these children, which is R3459.92. Thus, the cost of the CRP test outweighs the cost of oral amoxycillin or intravenous ampicillin, the antibiotics commonly used as empiric therapy for the treatment of community acquired childhood pneumonia. The results of the costs analysis in this study suggest that routine CRP testing is expensive in resource limited settings. However, more formal cost-effectiveness evaluations that take into account, for example, the costs of treating antibiotic related complications in viral pneumonia, are needed before any recommendations regarding CRP measurements are made.

Even if the CRP test spares antibiotic therapy in some children, it will not influence the duration of hospitalisation in any way. Hospitalised children usually require oxygen therapy and/or intravenous fluids. Once these children recover from their pneumonia and are feeding well and not requiring oxygen therapy, they are discharged home to complete their antibiotics orally (if still required). Thus the decision to send children home early (even if they do not require antibiotics) is based on clinical criteria and is not dependant on the CRP test result.

To minimise false-positive diagnoses, patients whose cultures were positive for skin flora (Table 4) were not considered to have proven bacterial pneumonia. However, these organisms may occasionally cause pneumonia. Ideally two separate blood cultures that yield the same organism and where the bacteraemia is related to the patient's illness should be considered proof of infection. A limitation of the present study is that a single blood culture was performed on the majority of patients. The microbiologists at the SAIMR reported the organisms listed in Table 4 as contaminants based on their antibiotic sensitivity patterns and growth on subsequent subcultures (M. Khoosal, personal communication). Those organisms regarded as contaminants have lower median CRP values compared to the median CRP values of the significant organisms. The median CRP values (25% - 75% nterquartile

range) of these contaminants were 26 (5.5 - 53) mg/L for those cases that were classified as viral pneumonia (Group 2) and 23 (10 - 73) mg/L for those patients regarded as having aetiologically undiagnosed pneumonia (Group 4) (Appendix 9). The inclusion of contaminant organisms into Groups 1 (bacterial pneumonia) and 3 (mixed pneumonia) would have: (a) decreased the median CRP value in these groups and (b) negatively influenced the statistical difference noted when the CRP values in the bacterial and viral pneumonia groups were compared.

The role of *Streptococcus viridans* as a cause of pneumonia in children is unclear. Definite infection (as defined by two positive blood cultures or a positive culture of lung aspirate material) has been documented in children with pneumonia. Suspected infection (defined as a single positive blood culture), when *S.viridans* may contribute to the child's illness, has been shown in childhood pneumonia. However many of these patients with a single positive blood culture had underlying neurological disorders (predisposing to aspiration pneumonia) or chronic pulmonary disorders.[42]

Viridans bacteraemia has also been documented in children where *S.viridans* is not thought to contribute to the patient's illness. These patients have had other well-documented infections (excluding pneumonia), where appropriate (or no) treatment of the primary illness resulted in a resolution of the patient's illness.[42] *S.viridans* is not a common cause of pneumonia in adults and about 50% of positive blood cultures are considered contaminants.[39] Thus, the analysis in the present study included and excluded *S.viridans* as a significant pathogen. Although the exclusion of *S.viridans* improved the ROC curves, it did not alter the clinical usefulness of the CRP as a screening test for bacterial pneumonia.

Previous studies investigating CRP values in children with pneumonia have shown a statistical difference when pneumonia (of any aetiology) was compared to asthma or

other lower respiratory tract infections such as 'acute bronchitis' and bronchiolitis.[13, 27] However, most of the studies that have compared mean CRP values in children with bacterial and viral pneumonia did not find a significant difference between the bacterial and viral groups.[13, 28, 43] The study by Babu *et al.* is the only report where significant differences were noted when mean CRP values were compared in bacterial and viral pneumonia.[27]

Some of these studies[28, 43] used serological diagnosis to confirm the diagnosis of bacterial pneumonia and the study by Isaacs included a small number (four) of patients with culture proven bacterial pneumonia.[13] Babu *et al.* were the only investigators that found statistical differences in the mean CRP values in children with bacterial pneumonia compared to viral pneumonia.[27] However, the criteria that Babu *et al.* used for the diagnosis for bacterial pneumonia were loosely based, relying solely on radiological criteria and not using either positive cultures or serological tests to verify bacterial or viral pneumonia.[27] Thus the interpretation of the findings in these studies can be difficult.[13, 27, 28, 43]

In contrast to previous studies,[13, 28 43] the present study has shown that the CRP values are significantly different in bacterial and viral pneumonia. The present study also found that threshold CRP values are statistically different in bacterial and viral pneumonia. The only previous study evaluating the use of threshold CRP values in bacterial and viral pneumonia reached a different conclusion. Nohynek *et al.* used threshold CRP values of 20-, 40-, 80- and 120-mg/L to distinguish between bacterial and viral pneumonia and found that these threshold values could not distinguish bacterial from viral pneumonia.[43]

What are the possible reasons that may account for the differing conclusions found in the present study? *S.pneumoniae* and *H.influenzae* caused the majority (65.7%) of bacterial pneumonia in the present study, a much higher percentage compared to

the previous studies.[13, 28 43] Studies have shown that the mean CRP concentration in childhood pneumonia caused by *S.pneumoniae* or *H.influenzae* is significantly higher compared to pneumonia caused by other bacteria or viruses.[43, 44] The findings of the present study are consistent with the above finding, the median CRP value associated with *S.pneumoniae* infections is 200mg/L and 110.5mg/L for *H.influenzae* infections. The higher median CRP values in HIV positive children with bacterial pneumonia may also be explained by the higher proportion of infections due to *S.pneumoniae* and *H.influenzae*. Thus, the overall significance of the median CRP values and the threshold CRP values seen in the present study was influenced by the following factors: firstly, the high CRP results seen in infections caused by *S.pneumoniae* and *H.influenzae* and secondly, the large proportion of bacterial pneumonia caused by these organisms.

ROC curve analyses have not been performed in other studies evaluating the use of CRP measurements in children with pneumonia. This form of analysis has been performed in a study evaluating CRP responses in neonatal sepsis.[41] In the above-mentioned study, a CRP value of ≥ 10mg/L was established as an appropriate threshold value above which results should be considered abnormal. However, this threshold value was calculated after serial CRP determinations were performed. A single CRP level performed at the beginning of an evaluation (for suspected neonatal sepsis) lacked sensitivity.[41]

The evaluation of the performance of a diagnostic test (such as the CRP) requires an objective and reliable method for identifying patients with and without the disease of interest,[41] but there are no simple criteria for the diagnosis of bacterial pneumonia in children. The most objective evidence of bacterial pneumonia is usually considered to be the recovery of a significant pathogen in the blood culture of a patient with radiologically confirmed pneumonia. The present analysis has only considered patients with positive blood cultures of established lower respiratory tract

pathogens as having bacterial pneumonia. Although it is estimated that only 7-20% of children with bacterial pneumonia have positive blood cultures, it would be expected that those with bacteraemia might have the highest CRP's, thus exaggerating the difference in CRP values between bacterial and viral pneumonia in this study.

Many studies have shown that CRP values are significantly different in bacterial and viral infections.[24, 26, 45] However, these studies have stopped short of making any recommendations with regard to antibiotic usage. In the present study, it is difficult to translate the statistical significance of CRP values in bacterial and viral pneumonia into clinical practice. A major reason for making the distinction between bacterial and viral pneumonia is to prevent unnecessary antibiotic usage, as the use of oxygen and other supportive therapy is largely decided on clinical grounds. The data shows that no threshold CRP value can be used to justify the withholding of antibiotic therapy in HIV uninfected children. In HIV infected children, where a CRP value of 10mg/L or more predicts all cases of bacterial pneumonia, an argument could be made for withholding antibiotics if the CRP value is less than 10mg/L. This is unlikely to influence clinical practice, as most clinicians would treat immunocompromised children presenting with pneumonia with antibiotics anyway, at least until blood cultures are proven sterile.

Ten percent of all children presenting with pneumonia in this study were severely malnourished (Table 2). In severely malnourished children antibiotics are often routinely prescribed, because of the high prevalence of concomitant infection. Routine antibiotic treatment reduces mortality in malnourished patients and is recommended as standard practice by the World Health Organisation.[46] CRP testing to determine initiation of antibiotic therapy in these children would be of no value.

The time factor has been suggested as an important reason for the low sensitivities seen in bacterial infections, as CRP levels may not be elevated within the first 12 hours of illness.[26, 32] At CHBH, only referred patients are seen. These patients are first seen at the community clinics, and if referred, have to wait for transport to bring them to hospital. At the hospital, they are first seen in the outpatient department and referred to the admission ward if needed. Although blood (for laboratory tests) may be drawn in the outpatient department, it is usually done in the admission ward. CRP concentrations should increase within 4 - 6 hours after the onset of inflammation or injury.[13] As the patients enrolled in this study are very unlikely to have had their CRP levels tested in this window period, reasons other than time delay may explain the poor CRP responses seen in some patients with bacterial infection.

When should a CRP test be performed? Clearly it is not a cost-effective investigation in developing countries, where the cost of empiric antibiotics (usually amoxycillin or co-trimoxazole) is cheaper than the cost of CRP testing. The ever-worsening epidemic of antibiotic resistance may create a situation where cheap antibiotics such as amoxycillin and co-trimoxazole prove to be of little value in the empiric treatment of suspected bacterial pneumonia. If this happens, expensive antibiotics will have to be used in countries that can afford them. In this scenario, serial CRP measurements, which have higher sensitivities and negative predictive values, may be used to assist in the decision to stop antibiotic therapy earlier. However, the clinical improvement of the patient is probably the major criterion that most clinicians employ in deciding when to stop antibiotic therapy.

The CRP test may be useful in patients who show no clinical improvement after antibiotic therapy is initiated and the physician considers changing antibiotic therapy. The rate of CRP decrease in children appropriately treated for their pneumonia is unknown and needs investigation. The negative predictive values of CRP

measurements in children with pneumonia have not been studied. Studies of CRP measurements in neonates have shown that CRP values have good negative predictive values, indicating when antibiotics may be safely stopped.[41] The present analysis shows similar results, and although suspected neonatal sepsis cannot be compared to community-acquired pneumonia, CRP measurements may be more helpful in influencing the decision to stop rather than initiate antibiotic therapy. The negative predictive value of serial CRP measurements in childhood nosocomial pneumonia needs investigation, especially if these measurements can be shown to stop expensive second- and third-line antibiotics earlier than usual.

The present study revealed that a threshold CRP value of 10mg/L in HIV infected children predicted all cases of bacterial pneumonia. This finding needs to be either confirmed or refuted in other studies. HIV infected children in developing countries usually suffer from several episodes of pneumonia – both bacterial and viral. Each episode of pneumonia is treated with antibiotics, which contributes to the development of antibiotic resistance. If antibiotic usage can be safely minimised in HIV infected children with pneumonia, the development of antibiotic resistance could possibly be retarded.

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Appendix 1 Number of patients according to group, CRP range value and HIV status

	Group 1 (Bacterial)			Group 2 (Viral)			Group 3 (Bacterial & viral)			Group 4 (Undetermined)		
CRP range (mg/L)	HIV+	HIV-	Total	HIV+	HIV-	Total	HIV+	HIV-	Total	HIV+	HIV-	Total
0-9	0	5	5	9	35	44	0	0	0	52	46	98
10-19	3	0	3	3	17	20	0	3	3	28	29	57
20-29	1	1	2	0	15	15	1	1	2	11	20	31
30-39	0	1	1	3	11	14	0	0	0	12	11	23
40-49	0	0	0	1	5	6	0	0	0	4	11	15
50-59	1	0	1	1	6	7	1	0	1	4	8	12
60-69	0	0	0	0	7	7	0	0	0	5	8	13
70-79	2	2	4	1	1	2	0	0	0	6	7	13
80-89	1	0	1	0	4	4	1	0	1	5	2	7
90-99	1	0	1	1	1	2	1	0	1	1	3	4
100-109	2	0	2	0	1	1	0	0	0	2	2	4
110-119	1	1	2	2	2	4	0	0	0	1	1	2
120-129	2	0	2	2	1	3	0	0	0	3	2	5
130-139	0	1	1	1	3	4	0	0	0	5	6	11
140-149	0	0	0	0	2	2	0	0	0	5	1	6
150-159	1	1	2	0	0	0	0	0	0	3	3	6
160-169	2	0	2	0	1	1	0	0	0	3	1	4
170-179	0	0	0	0	0	0	0	0	0	4	0	4
180-189	0	0	0	1	0	1	0	0	0	1	2	3
190-199	1	0	1	0	1	1	0	0	0	1	0	1
≥200	18	7	25	0	7	7	1	2	3	22	18	40
Total pat.'s	36	19	55	25	120	145	5	6	11	178	181	359
												570

Appendix 2. Sensitivities and specificities of CRP values predicting positive blood cultures in HIV-infected children.

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	100	30.05	69.95	22.40	100
≥20	92.68	45.32	54.68	25.50	96.84
≥30	87.80	50.74	49.26	26.47	95.37
≥40	87.80	58.13	41.87	29.75	95.93
≥50	87.80	60.59	39.41	31.03	96.09
≥60	82.93	63.05	36.95	31.19	94.81
≥70	82.93	65.52	34.48	32.69	95.00
≥80	78.05	68.97	31.03	33.68	93.96
≥90	73.17	71.43	28.57	34.09	92.95
≥100	68.29	72.41	27.59	33.33	91.88
≥110	63.41	73.40	26.60	32.50	90.85
≥120	60.98	74.88	25.12	32.89	90.48
≥130	56.10	77.34	22.66	33.33	89.71
≥140	56.10	80.30	19.70	36.51	90.06
≥150	56.10	82.76	17.24	39.66	90.32
≥160	53.66	84.24	15.76	40.74	90.00
≥170	48.78	85.71	14.63	40.82	89.23
≥180	48.78	87.68	12.32	44.44	89.45
≥190	48.78	88.67	11.33	46.51	89.55
≥200	46.34	89.16	10.84	46.34	89.16

Appendix 3. Sensitivities and specificities of CRP values predicting positive blood cultures in HIV-uninfected children.

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	80.00	26.91	73.09	9.09	94.19
≥20	68.00	42.19	57.81	8.90	94.07
≥30	60.00	53.82	46.18	9.74	94.19
≥40	56.00	61.13	38.87	10.69	94.36
≥50	56.00	66.45	33.55	12.17	94.79
≥60	56.00	71.10	28.90	13.86	95.11
≥70	56.00	76.08	23.20	16.28	95.42
≥80	48.00	78.74	21.26	15.79	94.80
≥90	48.00	80.73	19.27	17.14	94.92
≥100	48.00	82.06	17.94	18.18	95.00
≥110	48.00	83.06	16.94	19.05	95.06
≥120	44.00	84.05	15.95	18.64	94.76
≥130	44.00	85.05	14.95	19.64	94.81
≥140	40.00	88.04	11.96	21.74	94.64
≥150	40.00	89.04	10.96	23.26	94.70
≥160	36.00	90.03	9.97	23.08	94.43
≥170	36.00	90.70	9.30	24.32	94.46
≥180	36.00	90.70	9.30	24.32	94.46
≥190	36.00	91.36	8.64	25.71	94.50
≥200	36.00	91.69	8.31	26.47	94.52

Appendix 4. Sensitivities and specificities of CRP values predicting positive blood cultures in all children.

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	92.42	28.17	71.83	14.42	96.60
≥20	88.33	43.45	56.55	16.18	95.22
≥30	77.27	52.58	47.42	17.59	94.64
≥40	75.76	59.92	40.08	19.84	94.97
≥50	75.76	64.09	35.91	21.65	95.28
≥60	72.73	67.86	32.14	22.86	95.00
≥70	72.73	71.83	28.17	25.26	95.26
≥80	66.67	74.80	25.20	25.73	94.49
≥90	63.64	76.98	23.02	26.58	94.17
≥100	60.61	78.17	21.83	26.67	93.81
≥110	57.58	79.17	20.83	26.57	93.44
≥120	54.55	80.36	19.64	26.67	93.10
≥130	51.52	81.94	18.06	27.20	92.81
≥140	50.00	84.92	15.08	30.28	92.84
≥150	50.00	86.51	13.49	32.67	92.96
≥160	46.97	87.70	12.30	33.33	92.66
≥170	43.94	88.69	11.31	33.72	92.36
≥180	43.94	89.48	10.52	35.37	92.42
≥190	43.94	90.28	9.72	37.18	92.48
≥200	42.42	90.67	9.33	37.33	92.32

Appendix 5. Number of patients according to group, CRP range value and HIV status (*S.viridans* regarded as a contaminant)

	Group 1			Group 2			Group 3			Group 4		
CRP	HIV+	HIV-	Total	HIV+	HIV-	Total	HIV+	HIV-	Total	HIV+	HIV-	Total
0-9	0	2	2	9	35	44	0	0	0	52	49	101
10-19	2	0	2	3	17	20	0	3	3	29	29	58
20-29	0	1	1	0	15	15	1	1	2	12	20	32
30-39	0	1	1	3	11	14	0	0	0	12	11	23
40-49	0	0	0	1	5	6	0	0	0	4	11	15
50-59	1	0	1	2	6	8	0	0	0	4	8	12
60-69	0	0	0	0	7	7	0	0	0	5	8	13
70-79	2	2	4	1	1	2	0	0	0	6	7	13
80-89	1	0	1	0	4	4	1	0	1	5	2	7
90-99	1	0	1	1	1	2	1	0	1	1	3	4
100-109	2	0	2	0	1	1	0	0	0	2	2	4
110-119	1	1	2	2	2	4	0	0	0	1	1	2
120-129	2	0	2	2	1	3	0	0	0	3	2	5
130-139	0	1	1	1	3	4	0	0	0	5	6	11
140-149	0	0	0	0	2	2	0	0	0	5	1	6
150-159	1	1	2	0	0	0	0	0	0	3	3	6
160-169	2	0	2	0	1	1	0	0	0	3	1	4
170-179	0	0	0	0	0	0	0	0	0	4	0	4
180-189	0	0	0	1	0	1	0	0	0	1	2	3
190-199	1	0	1	0	1	1	0	0	0	1	0	1
≥200	18	7	25	0	7	7	1	2	3	22	18	40
Total pat.'s	43	16	50	26	120	146	4	6	10	180	184	364
												570

Appendix 6. Sensitivities and specificities of CRP levels predicting positive blood cultures in HIV-infected children (*S.viridans* regarded as a contaminant)

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	100	29.61	70.39	20.77	100
≥20	94.74	45.15	54.85	24.16	97.89
≥30	92.11	50.97	49.03	25.74	97.22
≥40	92.11	58.25	41.75	28.93	97.56
≥50	92.11	60.68	39.32	30.17	97.66
≥60	89.47	63.59	36.41	31.19	97.04
≥70	89.47	66.02	33.98	32.69	97.14
≥80	84.21	69.42	30.58	33.68	95.97
≥90	78.95	71.84	28.16	34.09	94.87
≥100	73.68	72.82	27.18	33.33	93.75
≥110	68.42	73.79	26.21	32.50	92.68
≥120	65.79	75.24	24.76	32.89	92.26
≥130	60.53	77.67	22.33	33.33	91.43
≥140	60.53	80.58	19.42	36.51	91.71
≥150	60.53	83.01	16.99	39.66	91.96
≥160	57.89	84.47	15.53	40.74	91.58
≥170	52.63	85.92	14.08	40.82	90.77
≥180	52.63	87.86	12.14	44.44	90.95
≥190	52.63	88.83	11.17	46.51	91.04
≥200	50.00	89.32	10.68	46.34	90.64

Appendix 7. Sensitivities and specificities of CRP levels predicting positive blood cultures in HIV-uninfected children (*S.viridans* regarded as a contaminant).

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	90.91	27.54	72.46	8.30	97.67
≥20	77.27	42.62	57.38	8.85	96.30
≥30	68.18	54.10	45.90	9.68	95.93
≥40	63.64	61.31	38.69	10.61	95.90
≥50	63.64	66.89	33.11	12.17	95.28
≥60	63.64	71.48	28.52	13.86	96.46
≥70	63.64	76.39	23.61	16.28	96.68
≥80	54.55	79.02	20.98	15.79	96.02
≥90	54.55	80.98	19.02	17.14	96.11
≥100	54.55	82.30	17.70	18.18	96.17
≥110	54.55	83.28	16.72	19.05	95.45
≥120	50.00	84.26	15.74	18.64	95.90
≥130	50.00	85.25	14.75	19.64	95.94
≥140	45.45	88.20	11.80	21.74	95.73
≥150	45.45	89.18	10.82	23.26	95.77
≥160	40.91	90.16	9.84	23.08	95.49
≥170	40.91	90.82	9.18	24.32	95.52
≥180	40.91	90.82	9.18	24.32	95.52
≥190	40.91	91.48	8.52	25.71	95.55
≥200	40.91	91.80	8.20	26.47	95.56

Appendix 8. Sensitivities and specificities of CRP levels predicting positive blood cultures in all children (*S.viridans* regarded as a contaminant).

CRP value	Sensitivity	Specificity	1-specificity	PPV	NPV
≥10	92.42	28.17	71.83	13.68	98.64
≥20	88.33	43.45	56.55	15.54	96.96
≥30	77.27	52.58	47.42	17.18	96.43
≥40	75.76	59.92	40.08	19.37	96.54
≥50	75.76	64.09	35.91	15.26	96.76
≥60	72.73	67.86	32.14	22.86	96.68
≥70	72.73	71.83	28.17	25.26	96.85
≥80	66.67	74.80	25.20	25.73	96.00
≥90	63.64	76.98	23.02	26.58	95.64
≥100	60.61	78.17	21.83	26.67	95.25
≥110	57.58	79.17	20.83	26.57	94.86
≥120	54.55	80.36	19.64	26.67	94.50
≥130	51.52	81.94	18.06	27.20	94.17
≥140	50.00	84.92	15.08	30.28	94.16
≥150	50.00	86.51	13.49	32.67	94.26
≥160	46.97	87.70	12.30	33.33	93.93
≥170	43.94	88.69	111.31	33.72	93.61
≥180	43.94	89.48	10.52	35.37	93.66
≥190	43.94	90.28	9.72	37.18	93.71
≥200	42.42	90.67	9.33	37.33	93.55

Appendix 9. Median CRP values and 25% - 75% interquartile ranges of all bacteria cultured on blood agar. Groups 2 and 4 (shaded) represent 'contaminant' bacteria.

Median (25% - 75% interquartile range) CRP in mg/L	
Group 1 - Total	160 (77 - 230)
Group 1 - HIV +	195 (99.5 - 234.5)
Group 1 - HIV -	113 (6 - 230)
Group 2 - Total	26 (5.5 - 53)
Group 2 - HIV +	7 (3 - 124)
Group 2 - HIV -	28 (7 - 50)
Group 3 - Total	56 (17 - 200)
Group 3 - HIV +	84 (56 - 90)
Group 3 - HIV -	22.5 (14 - 200)
Group 4 - Total	23 (10 - 73)
Group 4 - HIV +	22.5 (6 - 96.5)
Group 4 - HIV -	25 (13 - 72)

Celaya P.

Knowledge

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Pettipher C.A.

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