

**COMMUNITY ACQUIRED PNEUMONIA IN HIV AND NON-HIV INFECTED
ADULT PATIENTS PRESENTING TO A TEACHING HOSPITAL IN KWAZULU-
NATAL: AETIOLOGY, DISTRIBUTION AND DETERMINANTS OF
MORBIDITY AND MORTALITY**

by

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AUTHOR'S DECLARATION

This study represents original work by the author. It has not been submitted in any other form to the University. Where the use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Departments of Medicine, Microbiology, Anatomical Pathology, Virology, Nelson R Mandela School of Medicine, Faculty of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor UG Lalloo.

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I certify that the above statement is correct.

SIGNED: _____

PROFESSOR UG LALLOO

DEDICATION

To my family: Lee, Kudzai and Mudiwa

PRESENTATIONS ARISING FROM THIS DISSERTATION

1. **Nyamande K, Irusen EM, Chetty R, York D, Lalloo UG.** Evaluation of the polymerase chain reaction for *pneumocystis carinii* in mouth washings for the diagnosis of pneumocystis carinii pneumonia (PCP) in HIV infected patients - preliminary results. Faculty Research Day, September 2000.
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ABBREVIATIONS

rpm	revolutions per minute
S/CO	signal cut off units
T	thymidine
T lymphocyte	thymus derived lymphocyte
TMB	tetramethylbenzidine
TNF- α	tumour necrosis factor α
A	adenine
AIDS	acquired immunodeficiency syndrome
ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
BAL	bronchoalveolar lavage
B lymphocyte	bone marrow derived lymphocyte
°C	degrees Celsius
C	cytosine
CAP	community acquired pneumonia
CD4	cluster derivation 4
C5a	complement chemotactic peptide and 'a' denotes activated

EDTA	ethylenediaminetetraacetic acid
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
G	guanosine
Gp 120	glycoprotein 120
HIV	human immunodeficiency virus
HCl	hydrochloric acid
H ₂ O ₂	hydrogen peroxide
H ₂ SO ₄	sulphuric acid
H&E	haematoxylin and eosin
ICU	intensive care unit
Ig	immunoglobulin
IL	interleukin
IL-1 ra	interleukin 1 receptor antagonist
ILMA	immunoluminometric assay
ISR	immune status ratio
ITS	internal transcribed spacers
KCl	potassium chloride
kDA	kilo Dalton

LPS	lipopolysaccharide
M	molar
mM	millimole
min	minutes
ml	millilitre
mtr RNA	mitochondrial ribonucleic acid
NaCl	sodium chloride
Nm	nano metres
P1	primer 1
P2	primer 2
PaO ₂	partial pressure of arterial oxygen
PCP	pneumocystis pneumonia
PCR	polymerase chain reaction
PCT	procalcitonin

CHAPTER 1

INTRODUCTION

1.1 Definition of pneumonia

Pneumonia is defined as the accumulation of oedematous fluid and inflammatory cells in the alveoli in response to proliferation of micro organisms in normally sterile lung parenchyma (Fein et al, 1999). It is a syndrome characterised clinically by cough with or without sputum production, chest pain, fever, variable chest signs on physical examination and radiological evidence of a new pulmonary infiltrate or opacification (or consolidation to use a clinical term).

Community acquired pneumonia (CAP) is pneumonia acquired out of hospital. The candidate case should not have been hospitalised or resided in a long term care facility for more than 14 days prior to the onset of symptoms (Bartlett et al, 1998). Most studies have taken at least two of the following symptoms to indicate the presence of a lower respiratory tract infection: fever or hypothermia, rigors, sweats, new cough with or without sputum production, chest pain or the onset of dyspnoea. Non- specific symptoms may include fatigue, myalgias, abdominal pain, anorexia and headache.

Although it is generally assumed to be of acute or sub-acute onset, the issue of time has never been included in the definition of pneumonia in all major text books, review articles or land mark original papers focusing on the various aspects of the disease from aetiology to mortality. Thus in many publications, there are significant variations in the definition applied to pneumonia and there is no universally accepted time frame (Santiago et al 1998; Fein et al, 1999)

1.2 Pathogenesis of pneumonia

The approximate surface area of the lung is 70 square meters and it is normally sterile (West, 1995). Both the upper and lower airways have developed elaborate defence mechanisms to prevent the entry of organisms into the lung. The host becomes susceptible to infection of the respiratory tract if there is a breach in the integrity of any of these defence mechanisms (Nelson et al, 1995).

1.2.1 Defence mechanisms of the upper airways

Direct infection of the lung occurs via the inhalation route or through the aspiration of oropharyngeal secretions. The latter is the most common route. An elaborate defence system exists in the nasopharynx, the oropharynx and the conducting airways as outlined below. This defence system is made up of structural defenses, innate immunity and specific immune responses. Innate immunity consists of proteins (cell surface receptors, soluble substances) which identify noxious substances through recognition of carbohydrate structures. The innate system interacts with cellular and humoral factors such as alveolar macrophages, natural killer cells, B and T lymphocytes (Janeway, 1992; Medzhitox et al, 1996) to clear microbes from the airways.

Nasopharynx:

- (a) Air filtration. Particles larger than 10 μm are deposited in the nasal passages through inertia because of the curvature of the nasal turbinates and the narrow nasal ostia. Particles between 2-10 μm are deposited in the larger peripheral airways through inertia because of the frequent branching of the bronchial tree.

- (b) Mucociliary system. Ciliated nasal mucosa and mucous form a mucociliary barrier to pathogenic microbes deposited on the epithelial surface. Trapped organisms are expelled or swallowed.
- (c) Humoral defences such as IgA, IgG and complement proteins. Immunoglobulin has specific antibacterial and antiviral activity as well as opsonization.

Oropharynx:

- (a) Salivary flow. The mechanical flow of saliva over the epithelial surfaces assists in the movement and expulsion of trapped pathogenic organisms from the upper airways.
- (b) Normal microbial flow. There is microbial competition from the normal resident flora.
- (c) Epithelial cell turnover. The ability of microbes to adhere to surface glycoproteins is diminished by the high epithelial turnover
- (d) Mucosal pH. Alterations in mucosal pH affect the ability of potential pathogens to survive in a hostile microenvironment.
- (e) Bacterial binding site analogues. Alterations in the glycoprotein content of fibronectins and lectins may affect the pattern of bacterial adherence to the epithelium by altering availability of bacterial binding sites on the mucosal cells. Gram negative bacterial colonisation is thought to be facilitated by fibronectin, a large glycoprotein which alters the adherence properties of the host epithelium (Mason et al, 1990; Mason et al, 1991). IgA has specific antibacterial and antiviral properties. Gram negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia species*, *Proteus species* and

Klebsiella pneumoniae are capable of destroying IgA thus rendering the host more susceptible to pneumonia (Mulks et al, 1980; Milazzo et al, 1984).

Conducting airways:

- (a) epiglottis and glottis
- (b) cough reflex
- (c) airway branching

These form anatomical, structural and mechanical barriers to prevent macro and micro aspiration of oropharyngeal and gastric contents.

A number of cell-surface heparin sulphate-proteoglycans have been identified in airway epithelial cells (Lories et al, 1992; Maniscalco et al, 1992; David 1993). Heparin sulphate on cell surfaces may modulate growth factor receptor binding, influence cell adhesion to the matrix, influence the binding and regulation of a number of enzymes in the pericellular environment as well as in other crucial cell biological processes. There are two major families of heparin sulphate proteoglycans: the syndecan-like integral membrane heparin sulphate proteoglycans (SLIPs) and the glypican related integral membrane (GRIPs). Syndecans are important in the organization of epithelial cell sheets and in the maintenance of a non-thrombogenic endothelial surface (Lories et al, 1992; Maniscalco et al; 1992; David 1993; Roberts et al, 1997; Strieter et al, 1997). Both mechanisms are crucial to maintain intact defence. When heparin sulphate combines with IL-8, there is a significant rise in cytosolic free calcium and a four fold increase in neutrophil chemotaxis as compared with IL-8 alone (Webb et al, 1993). Specially developed materials that are heparin coated, are being manufactured to reduce microbial adhesion and biofilm formation on medical devices (Habash et al, 1999).

1.2.2 Defence mechanisms of the lower airway

The lower airways are defended by anatomic, mechanical, humoral and cellular mechanisms. These include the mucociliary system, IgA and IgG, surfactant and alveolar lining fluid, the alveolar macrophages, polymorphonuclear leukocytes, cytokines, oxygen free radicals and proteases. Infections with *Mycoplasma pneumoniae* and viruses destroy ciliary function. *Haemophilus influenzae* secretes substances that impair ciliary function (Afzelius, 1981).

Surfactant enhances phagocytosis of bacteria by alveolar macrophages (Hesse et al, 1988). Free fatty acids, lysozyme and iron binding proteins present in alveolar lining fluid possess antibacterial activity (Coonrod, 1986).

The alveolar macrophage is the cell primarily responsible for maintaining lung sterility. It can recruit polymorphonuclear cells via complement proteins such as C5a, leukotriene B₄, cytokines (TNF- α , IL-1 and IL-8) and bacterial cell wall components such as formyl-methionyl peptides or lipopolysaccharide (Shalaby et al, 1985; Klebanof et al, 1986; Lehrer et al, 1988; Baggiolini et al, 1989).

1.2.3 Pathophysiology of pneumonia in HIV infected subjects

Quantitative and functional abnormalities of immunity involving T cell function, B cell function and alteration in cytokine production occur at all stages of HIV infection (Noskin et al, 1994). CD4 cells are progressively depleted in the peripheral circulation leading to opportunistic infections with *Pneumocystis jirovecii* pneumonia as well as non-opportunistic infections with legionella, norcadia and rhodococcus. B lymphocyte

dysfunction leads to increased frequency and severity of infections due to *Streptococcus pneumoniae* and *Haemophilus influenzae* (Polsky et al, 1986; Selwyn et al, 1988). Defects in chemotaxis, phagocytosis and intracellular killing involving the monocyte macrophage system are responsible for susceptibility to infections due to *Staphylococcus aureus*, *Candida albicans* and *Toxoplasma gondii*. Neutrophil chemotaxis and killing is impaired and predisposes to infections with Gram negative bacilli, *Staphylococcus aureus* and *Candida albicans* (Nielsen et al, 1986; Ellis et al, 1988; Murphy et al, 1988). Local defence mechanisms that are impaired include function of macrophages, complement system and surfactant production (Beck et al, 1989). Emphysema-like pulmonary diseases associated with HIV infection have been described and may play a role in the impairment of local defences (Diaz et al, 1992).

1.3 Cytokines in CAP

The invasion of normally sterile lung triggers an immunological response characterised by involvement of a number of cytokines, complement proteins, macrophages, neutrophils, lymphocytes and other molecules of the immune system. Cytokines have multiple effects on the inflammatory response to infection. The main protagonists that drive the pro-inflammatory response are, TNF- α , IL-1 and IL-6. The major anti-inflammatory cytokines are IL-10 and IL-13. In the hierarchy of pro-inflammatory cytokine expression, TNF- α is released first, followed by IL-1 and then IL-6 (Fong et al, 1989; Baigrie et al, 1991).

1.3.1 Laboratory animal experimental data

The pulmonary inflammatory response to invading organisms has been extensively studied in rats. In the unchallenged lung, the levels of TNF- α are undetectable. There is a rapid increase in TNF levels on intratracheal instillation of bacterial lipopolysaccharide (LPS) followed by a marked inflammatory response due to migration of polymorphonuclear cells into the lung compartment. Thus TNF- α is a primary cytokine in the recruitment of inflammatory cells into the lung parenchyma (Ming et al, 1987; Nelson et al, 1989; Furie et al, 1989; Salyer et al, 1990; Nelson et al, 1991; Nelson et al, 1995). The administration of TNF- α soluble receptor virus to animals challenged with *Listeria monocytogenes* led to their rapid death compared with control animals which all survived. The authors concluded that TNF- α is a critical cytokine for adequate host defence in both the lung and the systemic compartment. TNF- α levels were found to peak in 90 minutes and return to undetectable levels by 3 hours. Long-term LPS injections led to the development of tolerance by the fifth consecutive day of administration, with the serum TNF α levels decreasing. Thus mononuclear phagocyte cytokine production may be down regulated after sepsis.

In other experiments, intratracheal LPS administration resulted in a high level of TNF α in BAL fluid without spill over into the vascular compartment (Haslett et al, 1987; Worthen et, 1987; Lily et al, 1989). TNF- α promotes lung host defence against Gram negative bacilli, *Staphylococcus aureus*, *Legionella pneumophila*, *Pneumocystis jirovecii* and *Pseudomonas aeruginosa*. The role of IL-1 in the lung response to LPS instilled intratracheally has been studied. IL-1 receptor antagonist (IL-1ra) inhibits LPS induced acute inflammation as measured by the number of neutrophils recovered in BAL fluid.

1.3.2 Cytokines in pneumonia: human data

TNF- α , IL-1 and IL-6 are recognised primary mediators of the early inflammatory response to bacterial invasion of the lung (Dehoux et al, 1994). They trigger the activation of immune cells, specific antibody production, recruitment and activation of monocytes and neutrophils and activation of endothelial cells. Measurement of TNF- α , IL-1 and IL-6 in BAL fluid, serum and alveolar macrophage and blood monocyte culture supernatants have shown compartmentalisation of the inflammatory response to infection in unilateral pneumonia (Dehoux et al, 1994).

Measurement of polymorphonuclear and monocyte function by chemiluminescence and of systemic cytokine levels in patients with CAP have shown activation on admission in 76% of patients and elevation of IL-6 and TNF- α at varying times of the illness (Moussa et al, 1994). This study did not show correlation between measurements of cytokine or phagocyte levels and outcome or indicators of disease severity.

Local lung and systemic expression of IL-1, IL-6 and TNF- α in severe pneumonia have been compared (Monton et al, 1999). The lung cytokine expression may be independent from the lung bacterial burden in the presence of antibiotic treatment. There does not seem to be a clear relationship between serum and BAL fluid cytokine levels and outcome.

Comparison of serum pro-inflammatory cytokine levels in patients with acute respiratory distress syndrome (ARDS) and severe pneumonia has been studied (Bauer et al, 2000). Serum TNF - α levels were significantly higher in ARDS patients than in patients with severe pneumonia. No statistically significant differences were observed for IL-1 and IL-6. IL-6 concentrations showed a large inter-individual variation and were not significantly

different between ARDS patients, those with severe pneumonia and controls. Attempts to associate cytokine concentrations with the prognosis have failed to establish a clear relationship. The biological effects are therefore difficult to interpret without taking into consideration the entire network of cytokines and other inflammatory mediators.

1.3.3 Cytokines in PCP and host pulmonary defence

The fungus *pneumocystis carinii* is now known as *Pneumocystis jirovecii* (Thomas et al, 2004). Although CD4 T lymphocytes are the primary defence against infection by *Pneumocystis jirovecii*, other mechanisms exist.

The alveolar macrophage can phagocytose and digest *Pneumocystis jirovecii* organisms by way of its interaction with the chemotactic factor major surface glycoprotein. The major surface glycoprotein can also induce an oxidative burst in alveolar macrophages (Hildalgo et al, 1991; Koziel et al, 1993; Pesanti, 1994). The binding of *Pneumocystis jirovecii* to alveolar macrophages occurs via mannose, fibronectin, Fc, and complement receptors. Alveolar macrophages release a number of cytokines following their binding to *Pneumocystis jirovecii*. TNF- α is released via alveolar macrophage interaction with beta-glucan cell wall components, vitronectin, fibronectin or antibodies directed against gp120 (Hoffman et al, 1993; Neese et al, 1994).

Interleukin-1 has direct effects against *Pneumocystis jirovecii* in vivo (Pesanti, 1991; Walzer, 1993). CD4 T lymphocytes produce interferon gamma which reduces the intensity of *Pneumocystis jirovecii* infection possibly through macrophage priming and activation as

well as augmented superoxide production and nitrogen oxide production (Soo Hoo et al, 1990; Beck et al, 1991; Simulian et al, 1994).

Evidence that humoral antibodies may have a role to play in defence against *Pneumocystis jirovecii* comes from data showing the development of *Pneumocystis jirovecii* pneumonia in B cell knockout mice that lack humoral immunity as well as in patients with common variable immunodeficiency and hypogammaglobulinaemia (Rao et al, 1983; Esolen et al, 1992; Lundgren, 1994; Sidman et al, 1994). Passively transferred *Pneumocystis jirovecii* antibodies can be partially protective against the organism (Walzer, 1993; Cushion, 1994). The humoral immune system may be involved in antigen presentation as well as in opsonization. As many as 43% of HIV infected patients with *Pneumocystis jirovecii* develop IgG antibodies directed against the major surface glycoprotein during the course of their infection (Lundgren et al, 1992). However, there is antigenic variation due to multiple genes encoding for major surface glycoproteins which are dispersed as tandem arrays on multiple chromosomes throughout the pneumocystis genome making it possible for the organism to elude host defences.

Concentrations of the pro-inflammatory cytokines have been studied in BAL fluid and serum of HIV infected patients with PCP (Perenboom et al, 1997). Concentrations of IL-1 were elevated in HIV positive patients with PCP compared with healthy subjects. Corticosteroid treatment had no effect on the concentrations of cytokines in BAL fluid or blood and did not suppress the production in alveolar cells. There was no correlation between cytokine production and the severity of the infection. The pro-inflammatory reaction was evident in BAL while the antiinflammatory response was dominant in the circulation as measured by IL-1 ra.

1.4 Epidemiology of CAP

1.4.1 Incidence in the immunocompetent

Data on the incidence of CAP has come mostly from the developed countries. Between 2 and 4 million cases of CAP are seen in the United States every year. Estimates put the incidence at 10 - 12 cases per 1,000 persons per year (Foy et al, 1973; Marie 1994; Mandell, 1995). The incidence is highest at the extremes of age (Foy et al 1979; Jokinen et al, 1993; Mandell, 1995). Studies from the USA and Finland suggest attack rates of 12 - 18 and 35 per 1,000 population per year respectively in those below the age of 5 years. In patients over 75 years of age, rates above 30 cases per 1,000 population per year are seen. The incidence is lowest in the 5-60 year age groups where it is reported to be between 1 and 5 per 1,000 population per year.

1.4.2 Incidence in HIV infected subjects

The marked increase in the number of patients infected with HIV has created a subset of patients with CAP who, in addition to having pneumonia due to opportunistic pathogens, may present with severe or atypical manifestations of infection with conventional pathogens. CAP as a complication of HIV can occur at any level of CD4 count but becomes increasingly frequent as the CD4 count declines (Hirschitnik et al, 1995). There is no accurate data on the prevalence and incidence of CAP in HIV, due to a lack of controlled studies and differing diagnostic criteria (Noskin et al, 1996).

The Prospective Study of Pulmonary Complications of HIV Infection looked at the incidence of respiratory tract infections in 1116 HIV seropositive subjects and compared their findings simultaneously to 165 non-HIV infected patients over an 18 month follow up

period (Wallace et al, 1993). The most frequent respiratory diagnoses in HIV seropositive subjects were upper respiratory tract infection (33.4%), acute bronchitis (16.0%), acute sinusitis (5.3%) and bacterial pneumonia (4.8%). PCP was seen in 3.9% of patients. The results of this study are summarized in Table 1.1.

Table 1.1 Prospective Study of Pulmonary Complications in HIV (Wallace et al, 1993)

		HIV positive (n=1116)	HIV negative (n=165)
Pulmonary infections			
Opportunistic	<i>P jirovecii</i>	43 (3.9%)*	0
	<i>Streptococcus pneumoniae</i>	5 (0.5%)	0
	<i>Cytomegalovirus</i>	3 (0.3%)	0
	<i>Herpes simplex virus</i>	1 (0.1%)	0
	<i>Toxoplasma gondii</i>	1 (0.1%)	0
	Non-tuberculous mycobacteria	12 (1.1%)	0
Nonopportunistic	Bacterial pneumonia syndrome	53 (4.8%)*	1 (0.6%)
	Unspecified pneumonitis	8 (0.7%)	1 (0.6%)
	<i>M. tuberculosis</i>	10 (0.9%)	0
	<i>Histoplasma capsulatum</i>	1 (0.1%)	0
	Lung abscess	1 (0.1%)	0
	Any pulmonary infection	115 (10.3%)*	2 (1.2%)
Pulmonary neoplasms	Kaposi's sarcoma	2 (0.2%)	0
	Carcinoma	3 (0.3%)	0
	Any pulmonary neoplasm	5 (0.5%)	0
Idiopathic pulmonary processes	Nonspecific interstitial pneumonitis	8 (0.7%)	0
Miscellaneous pulmonary events	Pulmonary embolus	1 (0.1%)	0
	Pneumothorax	1 (0.1%)	1 (0.6%)
	Congestive heart failure	2 (0.2%)	0
	Any miscellaneous pulmonary event	4 (0.4%)	1 (0.6%)
Ambulatory respiratory illnesses	Upper respiratory infection	373 (33.4%)	42 (25.5%)
	Bronchitis	179 (16.0%)*	15 (9.1%)
	Sinusitis	59 (5.3%)*	3 (1.8%)
	Influenza syndrome	47 (4.2%)	7 (4.2%)
	Otitis media	25 (2.2%)	1 (0.6%)
	Streptococcal pharyngitis	18 (1.6%)	2 (1.2%)
	Any ambulatory respiratory illness	522 (46.8%)	56 (33.9%)

p< 0.05 compared with HIV

In South Africa, pneumonia was a common cause of death particularly among children and the elderly before the era of HIV (Benatar et al, 1985). In 1984, 5 % of all deaths in the population group above the age of 65 were due to CAP. However, the demography of disease in sub-Saharan Africa has been altered dramatically by the HIV epidemic with the

region reporting the highest infection rates and with a large reservoir of infected but asymptomatic subjects.

1.5 Aetiology of CAP

Prospective studies of the aetiology of CAP have failed to identify the cause in 40% to 60% of cases (Marie TJ, 1994). The prior use of antibiotics before investigations are performed may be as high as 30 % (Marie TJ, 1994) and this may account for a substantial number of CAP where the aetiology is unknown. Viruses may also account for some cases. Viruses are not readily cultured from sputum and the use of acute and convalescent phase serum is usually available too late to be of any value in the management of patients.

Cost is also prohibitive and viral pneumonias are self limiting. Thus, the exclusion of viral tests in studies addressing the aetiology of CAP has some justification. More than one organism has been identified in 2% - 5% of patients with CAP (Marrie et al, 1989; Fang et al, 1990; The British Thoracic Society, 1993; Mundy et al, 1995; Keller et al, 1995; Marston et al, 1997).

Radiographically, CAP has traditionally been subdivided into lobar, bronchopneumonia and interstitial types (Heitzman, 1989). The radiographic pattern cannot be used to predict the causative organism or to differentiate among causative groups of pathogens. This data has come from several studies (Tew et al, 1977; MacFarlane et al, 1984; Farr et al, 1989). In addition, interobserver variability and individual performances exist in the interpretation of chest radiographs (Melbye et al, 1992; Albaum et al, 1996; Katz et al, 1999).

1.5.1 Aetiology of CAP in the immunocompetent

The microbial patterns reported have differed considerably. Possible explanations are:

- (a) Region studied
- (b) Patient population included
- (c) Extent of microbiologic testing
- (d) Types of microbiologic techniques
- (e) Prior antibiotic use which may mask the identity of a pathogen
- (f) Whether or not specialized investigations for atypical organisms were undertaken
- (g) Ability of patients to produce sputum samples and whether the samples were of good quality
- (h) Scientific and technical ability to pick up new pathogens
- (i) The emergence of HIV disease as a major cause of immune dysfunction
- (j) Recognition that it is not possible to perform all known microbiological tests due to logistical problems, cost and technical expertise.

To date, studies have consistently shown *Streptococcus pneumoniae* to be the commonest cause of CAP in patients in whom an organism was isolated (Table 1.2). Other common pathogens include *Haemophilus influenzae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*.

Streptococcus pneumoniae

The incidence rates reported for pneumococcus have been reported as 34% (UK), 46% (Sweden), 14% (Spain), 15% (USA) and 8% (Canada) (Marrie et al, 1989; Fang et al 1990; Ortvist et al, 1990; Blanquer et al, 1991, Mandell, 1995). Pneumococcal bacteraemia

occurs in 13% to 40% and is more likely in patients with co-morbid disease. Mortality rates for bacteremic patients can be as high as 19.5% (Austrian et al, 1964; Marrie, 1992).

Haemophilus influenzae

The incidence of CAP due to *Haemophilus influenzae* is 2% to 11% with higher rates in the elderly and patients with comorbid disease (Fang et al, 1990; Macfarlane, 1994). In bacteremic *Haemophilus influenzae* pneumonia, mortality may be as high as 57% (Wallace et al, 1978).

Moraxella catarrhalis

Less than 2% of cases of CAP are attributable to *Moraxella catarrhalis*. Patients with coexisting lung disease are more prone to infection with this organism.

Legionella pneumophila

Of the 34 species of legionella, *Legionella pneumophila* is the species most commonly associated with pneumonia (90% of cases). Legionella accounts for 1% to 16% of cases of CAP (Muder et al, 1983; Woodhead et al, 1987) The sporadic form is more common than the epidemic form. Mortality in legionella pneumonia averages 19% and ranges from 5% to 80% in compromised patients with underlying lung disease, smokers, the elderly and patients receiving corticosteroids and other immunosuppressive drugs (England et al, 1981).

Mycoplasma pneumoniae

Mycoplasma pneumoniae accounts for 4% to 51% of cases of CAP depending on whether or not there is epidemic activity and the diagnostic tests performed (Murray et al, 1975;

Atmar et al, 1989). Pneumonia due to *Mycoplasma pneumoniae* is generally not severe but life-threatening cases have been reported.

Chlamydia pneumoniae (TWAR)

Chlamydia pneumoniae is a cause of sporadic and epidemic pneumonia worldwide. The incidence is between 6% (an elderly Canadian population) and 15% (a Spanish study) (Almirall et al, 1993). Although the majority of cases are mild to moderate, severe cases have been reported.

Aerobic Gram negative bacilli

Escherichia coli, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are causes of CAP in the elderly, those with comorbid illness and in patients with severe CAP requiring admission to intensive care. In a study from the UK (British Thoracic Society, 1987), these pathogens accounted for less than 1% of cases in 453 hospitalized adults with CAP. The incidence rates reported from other countries are 5.9% (United States), 15% (France) and 25% from Spain (Fang et al, 1990; Pachon et al, 1990; Moine et al, 1994). The mortality rate in pneumonia due to Gram negative bacilli averages 33% (Gilbert et al, 1994).

Staphylococcus aureus

Most studies have shown *Staphylococcus aureus* to be an uncommon cause of CAP (Sopena et al, 1999). In contrast, the mortality is high. Median mortality rates of 39% have been reported (Gilbert et al, 1994; Fine et al, 1990).

1.5.2. CAP in HIV infected subjects

The Prospective Study of Pulmonary Complications of HIV Infection examined the types of respiratory disorders that occur across the full range of HIV disease. *Pneumocystis jirovecii* and bacterial pneumonia syndrome were found to be the most frequent pulmonary infections (Wallace et al, 1993), with the latter being the most frequently encountered. The risk of bacterial pneumonia in the HIV infected compared with the seronegative control was found to be 7.8 higher. The spectrum of pathogens isolated included *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Klebsiella pneumoniae*, *Streptococcus sanguinii*, and *Pseudomonas aeruginosa*. Bacterial pneumonia occurs at all CD4 counts but becomes more frequent as the CD4 count declines (Hirschtik et al, 1995). *Streptococcus pneumoniae* is the commonest bacterial cause of CAP in HIV disease (Witt et al, 1987; Magnenat et al, 1991; Miller et al, 1994; Noskin et al, 1996). However, some studies have documented *Haemophilus influenzae* as the leading pathogen (Polsky et al, 1986). Two of the studies are summarized in tables 1.3 ,1.4 and 1.5. Mundy et al (1995) found a high incidence of pneumococcus and PCP in 180 HIV infected patients with CAP (Table 1.6).

Site	Date	No. of cases diagnosed aetiologically	First	Second	Third	Fourth	Fifth	Unknown aetiology (%)
Barcelona (Spain; Fine MJ et al, 1996.)	1994-1996	389	<i>S pneumoniae</i> (24)	<i>C pneumoniae</i> (14)	Legionella spp (13)	<i>H influenzae</i> (2)	<i>P aeruginosa</i> (2)	42
Okayama (Japan; Ishida T et al, 1998.)	1994-1997	326	<i>S pneumoniae</i> (23)	<i>H influenzae</i> (7)	<i>M pneumoniae</i> (5)	<i>K pneumoniae</i> (4)	<i>Streptococcus miller</i> (4)	39
Verona (Italy; Guglielmo L et al, 1997)	NA	345	Gram negative rods (8)	<i>M pneumoniae</i> (4)	Legionella spp (3)	Viral (3)	<i>S pneumoniae</i> (2)	NA
Ohio (USA; Marston BJ et al, 1997)	1991	2776	<i>M pneumoniae</i> (33)	<i>S pneumoniae</i> (13)	<i>C pneumoniae</i> (9)	Influenza A (7)	<i>H influenzae</i> (7)	20
Beer-Sheva (Israel; Lieberman D et al, 1996)	1991-1992	346	<i>S pneumoniae</i> (43)	<i>M pneumoniae</i> (29)	<i>C pneumoniae</i> (18)	Legionella spp (16)	Viral (10)	19
Murcia (Spain; Gomez J et al, 1996)	1991-1994	100	<i>S pneumoniae</i> (23)	<i>C pneumoniae</i> (21)	<i>H influenzae</i> (19)	Legionella spp (15)	<i>M pneumoniae</i> (11)	NA
Leiden (The Netherlands; Bohte R et al, 1995)	1991-1993	334	<i>Streptococcus pneumoniae</i> (27)	<i>Haemophilus influenzae</i> (8)	<i>M pneumoniae</i> (6)	<i>Chlamydia pneumoniae</i> (3)	Legionella spp (2)	45
Four U.S.A. cities (Vergis EN et al, 2000)	1994-1996	149	<i>Streptococcus pneumoniae</i> (25)	Legionella spp (14)	<i>Haemophilus influenzae</i> (13)	<i>C pneumoniae</i> (10)	<i>Mycoplasma pneumoniae</i> (9)	41

Table 1.2 Aetiology of CAP in selected studies that had more than 100 hospitalized patients

Table 1.3 Bacterial diagnosis in 49 episodes of community acquired lobar pneumonia in 45 HIV positive patients (Adapted from Miller et al, 1994). Middlesex, London, UK

Organism	n
<i>Streptococcus pneumoniae</i>	11
1 co infected with <i>staphylococcus epidermidis</i>	
1 co infected with <i>haemophilus influenzae</i>	
and <i>staphylococcus aureus</i>	
<i>Staphylococcus aureus</i>	5
1 co infected with <i>pneumocystis jirovecii</i>	
1 coinfectd with Group A Streptococcus	
<i>Pseudomonas aeruginosa</i>	2
<i>Pneumocystis jirovecii</i>	2
1 co infected with <i>enterobacter cloacae</i>	
<i>Haemophilus influenzae</i>	2
1 co infected with <i>salmonella typhymurium</i>	
1 co infected with <i>mycobacterium avium-intracellulare</i>	
<i>Fusobacterium nucleatum</i>	1
<i>Salmonella cholera-suis</i>	1
<i>Mycobacterium tuberculosis</i>	1
No organism identified	24

Table 1.4 Clinical diagnoses in 101 HIV infected patients (Magnenat et al, 1991).

Geneva, Switzerland

Diagnosis	Episodes (%)	Patients
Bacterial pneumonia	60(45.5)	45
Pneumocystis pneumonia	36(27)	33
Mycobacteriosis	11(8.3)	8
Kaposi's sarcoma	9(7)	4
Bronchitis	6(5)	3
Lymphoma	3(2.1)	3
Primary pulmonary hypertension	3(2.1)	3
Others	4(3)	2
Total	132	101

Table 1.5 Bacterial cultures in 60 episodes of bacterial pneumonia in 45 HIV infected patients (Magnenat et al, 1991). Geneva, Switzerland

Bacteria	Bronchoscopy (n=37)			No Bronchoscopy (n=23)	
	Site of cultures			Site of cultures	
	BAL	Sputum	Blood	Sputum	Blood
<i>Streptococcus pneumoniae</i>	12(8)	7(2)	-	3(3)	7(4)
<i>Haemophilus influenzae</i>	4(3)	2(2)	-	1(1)	-
<i>Branhamella catarrhalis</i>	2(2)	-	5(1)	-	-
<i>Streptococcus milleri</i>	2(2)	-	-	-	-
<i>Klebsiella pneumoniae</i>	1(1)	-	-	1	-
<i>Streptococcus species</i>	2(1)	-	-	-	-
<i>Staphylococcus aureus</i>	1	-	-	-	-
<i>Pseudomonas aeruginosa</i>	1	-	-	-	-
<i>Corynebacterium</i>	1	-	-	-	-
Mixed flora	2(1)	-	-	1	-
Total	28(18)	9(4)	5(1)	6(4)	7(4)
No organism		9		11	

Other bacteria that cause pneumonia in HIV disease include legionella (Murray et al, 1984; Cohn et al, 1991; Hirschtik et al, 1995), *Streptococcus glaciata*, *Rhodococcus species* and *Nocardia species* (Rodriguez et al, 1986; Telzak et al, 1989).

Mortality from HIV associated CAP in developed countries is considerable. The mortality ranges from 2.6 % to 27 % in hospital or after four weeks (Teira et al, 1992; Falco et al, 1994; Boschinni et al, 1996; Tumbarello et al, 1998; Cordero et al, 2000).

Table 1.6 Aetiology of pneumonia in 180 HIV infected hospitalized patients (Mundy LM et al, 1995). Maryland, USA.

Diagnosis	Definitive	Presumptive	Total (%)
<i>Streptococcus pneumoniae</i>	18	20	38 (21.1)
<i>Pneumocystis jirovecii</i>	47	1	48 (26.7)
<i>Haemophilus influenzae</i>	1	12	13 (7.2)
Gram negative bacilli	6	5	11 (6.1)
Viral	7	0	7 (3.9)
<i>Chlamydia pneumoniae</i>	7	0	7 (3.9)
<i>S aureus</i>	2	5	7 (3.9)
<i>L pneumophilla</i>	5	1	6 (3.3)
<i>M tuberculosis</i>	4	0	4 (2.2)
<i>M catarrhalis</i>	0	2	2 (1.1)
<i>M pneumoniae</i>	10	0	1 (0.5)
Fungal	2	0	2 (1.1)
Other	2	2	4 (2.2)
Aspiration	0	5	5 (2.8)
Unknown	0	46	46 (25.6)
Total	103	99	202

1.5.3 Aetiology of CAP: a review of studies from sub-Saharan Africa

Studies of the aetiology of CAP in sub-Saharan Africa are few. Prout and colleagues studied 81 patients with CAP at Groote Schuur hospital over a 5-month period from February to June 1980. An aetiological agent was identified in 54 % of patients with bacterial agents being found in 37 % of patients, mycoplasma and viral infections in 21 % and tuberculosis in 6 %. Twelve percent of patients had coinfections. In the subjects in who a pathogen was identified, *Streptococcus pneumoniae* was isolated in 63 %, *Haemophilus influenzae* in 26.7%, *Staphylococcus aureus* in 6.7% and other gram negative organisms in 10 %. No microbiological tests were performed for chlamydia and legionella species. This study was done before the era of HIV infection, highlighting the importance of regular studies to guide therapy. Maartens et al, in a study conducted at Groote Schuur from 1987 to 1988, prospectively looked at the proportion of cases of CAP caused by

chlamydia, legionella, coxiella and mycoplasma. Thirty-two of 92 (35.9 %) patients were found to be infected with atypical pathogens, the commonest being *Chlamydia pneumoniae* (20.7 %), and *Legionella pneumophila* (8.7 %).

1.5.4 The effect of HIV infection on the epidemiology of CAP in Africa

There are no good studies, in contrast to North America and Western Europe. HIV infected patients have been excluded from previous epidemiological studies of CAP (Mundy et al, 1995).

Two studies were conducted in Cameroon and Ethiopia in the 1990s (Koulla Shiro et al, 1996; Aderaye, 1994). In the study from Cameroon, 110 patients were studied of which 25% were HIV infected. There were no differences in aetiology and outcome between HIV seropositive and HIV seronegative patients. Mortality was the same in the two groups. *Streptococcus pneumoniae* was the commonest pathogen and no cases of PCP were reported. There is no mention of *Mycobacterium tuberculosis* (Table 1.7).

Table 1.7 Bacterial aetiology of CAP according to HIV sero-status of 110 patients
(Koulla-Shiro S et al, 1996) Cameroon

Organism	Frequency (%) HIV negative n=82	Frequency (%) HIV positive n=28	p value
<i>Streptococcus pneumoniae</i>	24 (29.3)	7 (25)	NS
<i>Mycoplasma pneumoniae</i>	6 (8.8)	3 (12.0)	NS
<i>Coxiella burnetti</i>	6 (8.8)	0	
<i>Klebsiella pneumoniae</i>	5 (6.1)	0	
<i>Chlamydia pneumoniae</i>	3 (4.4)	1 (4.0)	NS
<i>Staphylococcus aureus</i>	1 (1.2)	3 (10.7)	NS
<i>Haemophilus influenzae</i>	1 (1.2)	1 (3.6)	NS
<i>Streptococcus pyogenes</i>	2 (2.4)	0	
<i>Moraxella catarrhalis</i>	1 (1.2)	0	
Unknown	39 (47.6)	14 (50)	NS
Total	88	29	

The Ethiopian study (Aderaye, 1994) had only 9 HIV seropositive patients and thus the numbers were too small to make any meaningful comparison. From Cote d'Ivoire, Grant et al (1998) looked at the spectrum of disease among HIV infected adults hospitalized in a respiratory medicine unit in Abidjan. Seventy five percent of patients were HIV seropositive with the most frequent diagnosis being tuberculosis (61%) followed by bacterial pneumonia (15%), Gram negative septicaemia (9%) and empyema (5%). Most HIV seropositive patients had CD4 counts consistent with advanced disease. In- hospital mortality was 27% for HIV seropositive patients and 22% for HIV seronegative patients ($p = 0.5$). The strongest independent risk factors for death were cachexia, male sex and age over 40 years. Thus malnutrition and cachexia are risk factors for mortality. A necropsy study from Cote d'Ivoire documenting the range of disease in

African children infected with HIV (Lucas et al, 1996) found a high prevalence of PCP (31 %) and a low prevalence of tuberculosis (1/78). A similar study from Zimbabwe (Ikeogu et al, 1996) looked at the pulmonary manifestations in HIV seropositive and malnourished children who died at home. PCP was present in 16 %, CMV pneumonia in 7 %, and lymphoid interstitial pneumonitis in 9 %. In two surveys from Cote d'Ivoire (Lucas et al, 1993) and Harare, *Mycobacterium tuberculosis* accounted for 32 % and 50 % of deaths in adults with HIV infection.

1.6 Evaluation of the severity of CAP

There is no universally accepted definition for severe CAP. Different criteria have been published. As shown in table 1.8, there are areas of agreement and disagreement in the assessment of severity criteria published by the ATS, BTS, CTS and the Republic of South Africa (Feldman, 1998).

Mortality is high in patients with severe pneumonia. If one or more of the factors listed below are present CAP is defined as severe and the mortality can be as high as 50% (Ewig et al, 1998; Cazolla et al, 2001).

1. Respiratory rate greater than 30 breaths per minute
2. $\text{PaO}_2/\text{FiO}_2$ ratio < 250
3. Rapid radiographic worsening (> 50 % increase in infiltrate size within 48 h)
4. Bilateral or multilobar involvement
5. Shock
6. Need for vasopressors for more than 4 hours
7. Evidence of sepsis with organ dysfunction

The sensitivity, specificity, positive and negative predictive values of the criteria used to define CAP varied. Defining pneumonia according to the ATS guidelines has a high sensitivity of 98 % but the specificity and positive predictive value are only 32 % and 24 % respectively (Ewig et al, 1998). The definition of pneumonia impacts on the data on CAP severity.

A prediction rule to identify patients with severe CAP was derived by Fine et al (1997) in a landmark study that retrospectively analyzed data from 14 199 patients from a 78 hospital computerized data base. Patients with HIV disease were excluded in the derivation of the Fine score.

Table 1.8 Assessment of severity of pneumonia in hospitalised cases with CAP
(Adapted from Feldman, 1998)

Features	ATS	BTS	CTS	RSA
Age > 60 years	0	+	0	+
Underlying co-morbidity	0	+	0	+
Confusion/decreased consciousness	0	+	0	+
Atrial fibrillation	0	+	0	0
Respiratory rate >30 bpm	+	+	+	+
Shock				
Systolic BP < 90mmHg	+	0	0	+
Diastolic BP < 60mmHg	+	+	0	+
Need for vasopressors for > 4 hours	+	0	0	+
Respiratory failure				
PaO ₂ < 60mmHg	0	+	+	+
PaO ₂ /FiO ₂ ratio < 250 mmHg	+	0	0	0
Sepsis with organ dysfunction	0	0	+	0
Extra thoracic septic complications	0	0	+	+
Laboratory data				
White cell count <4 or >30 x 10 ⁹ /l	0	+	0	+
Albumin < 30g/l	0	+	0	+
bacteraemia	0	+	0	0
Renal dysfunction				
low urine output	+	0	0	0
acute renal failure	+	0	0	+
Urea > 7 mmol/l	0	+	0	+
Radiology				
multilobar consolidation	+	+	+	+
cavitation	0	0	+	+
Need for mechanical ventilation	+	0	0	+

0 = parameter was not mentioned.

ATS = one or more of the features

BTS = one or more of the features

CTS = general guidelines only

RSA = two or more parameters

Validation of the ATS severity criteria for CAP was studied in a cohort of patients from Spain (Cordero et al, 2000). Three factors were independently related to mortality:

1. CD4 count < 100.
2. Radiographic progression of disease
3. Shock.

The authors acknowledged that further independent studies are required.

1.7. Procalcitonin

Procalcitonin is the propeptide of the hormone calcitonin, which is normally produced by the C cells of the thyroid gland. It has been touted as a new marker of the inflammatory response to infection (Reinhart et al, 2000).

1.7.1 History and physiology of PCT

The interest in PCT as a marker of inflammation emanated from the institute G Roussy in France in the last decade when the molecule was found to be markedly elevated in septic burns patients (Reihart et al, 2000).

PCT is a 116 amino acid polypeptide molecule (Rau et al, 2000). At its amino terminus, it contains a peptide termed nPCT (or PAS-57) which is a dibasic amino acid cleavage site at the amino terminus of calcitonin (a 32 amino acid part of the prohormone). PCT has a molecular weight of 13 kDA and lacks hormonal activity (Hammer et al, 2000). Its biological function is unknown. The site of production is unknown but neuroendocrine cells that produce procalcitonin have been found in liver and lung tissue (Reith et al, 2000).

PCT is not released into the circulation in healthy subjects (Reinhart et al, 2000), hence plasma levels are very low. Although fundamental aspects of PCT in health and disease are still being studied, it has been established that the stimuli for its release are endotoxin, TNF α , exotoxin and as yet unidentified costimulatory factors. The cascade of inflammatory cytokines that are released during systemic infection damage the tight junctions that bind together the inner and outer portions of the cell membranes of neuroendocrine cells. Mature hormone is secreted by apical cell membranes while basolateral membranes secrete incompletely processed hormone. When a cell loses its polarity, the entire cell membrane converts into a basolateral membrane resulting in the Golgi apparatus receiving messages to produce predominantly nonprocessed or incompletely processed procalcitonin.

When endotoxin is injected into volunteers, PCT appears in blood after 3 hours, plateaus at 6 hours and remains elevated for 24 hours. After a known stimulus, the appearance of PCT in the circulation is slightly slower (2 – 4 hours) than IL – 6 (1 hour) but faster than C-reactive protein (24 hours).

The half-life of PCT is 24 hours and this is not altered during renal failure when compared to patients with normal renal function. There is no correlation with creatinine clearance, age or gender (Meisner et al, 2000). The molecule is chemically stable in plasma and is not converted to calcitonin. PCT is cleaved while circulating in the periphery by specific proteases to calcitonin, katalcin, and an N-terminal residue. The pharmacokinetic properties of PCT enable it to serve as an early as well as late (24 hours and beyond) marker of infection and inflammation. When measured on a daily basis, PCT remains

detectable (due to its longer half-life) when markers such as TNF- α and IL-6 are no longer detectable.

1.7.2 Procalcitonin in infection and sepsis

Studies of PCT have focused on its utilization in discriminating infectious from non-infectious causes of inflammation, and as a marker of severe sepsis in critically ill patients in intensive care (Renhart K et al, 2000). In one of the early studies from Bangkok (Smith et al, 1995) PCT levels correlated with the severity of illness in meliodosis. In a cohort of intensive care patients (Brunkhorst et al, 2000), PCT levels were found to be highest in patients with septic shock. The levels correlated with the degree of sepsis. PCT may be better than the Apache 11 score in discriminating between sepsis and severe sepsis. Although the appearance of PCT was related to the appearance and severity of bacterial infection, it did not differentiate the causative micro organism (Cherval et al, 2000). The PCT levels correlated closely with the levels of other inflammatory markers such as TNF- α , IL-6 and IL-8.

Procalcitonin levels are higher in patients with infected pancreatic necrosis than in those with sterile necrosis. PCT is not detectable in healthy subjects and the levels are only moderately elevated in non-septic inflammatory processes, various metastatic cancers and with minor infection (Rau et al, 2000). Little or no elevation of PCT is found in viral infections (including HIV) but levels increase early during systemic bacterial and fungal infections and decrease when the infection resolves. The role of PCT in differentiating pneumonia due to common bacteria, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* needs to be studied.

1.8 Blood cultures in CAP

The utility and cost effectiveness of blood cultures in CAP is still debatable. The protagonists of routine blood cultures in hospitalised patients with CAP have put forward several arguments in favour of the practice:

1. Positive blood cultures remain the “gold standard for the diagnosis of pneumococcal and other pneumonias.
2. Blood cultures can be useful in tailoring appropriate treatment in certain cases.
3. Bacteraemia is a risk factor for poor outcome.
4. Susceptibility data obtained from pneumococcal isolates are of crucial importance in guiding therapy and monitoring resistance.
5. Documenting high risk pathogens such as *Staphylococcus aureus* and gram negative bacilli which are associated with higher morbidity and mortality.

There are equally strong arguments against obtaining blood cultures in all hospitalized patients with CAP:

1. The yield of blood cultures is low ranging from 4 % and at best 18 %.
Explanations include prior use of antibiotics and lack of bacteraemia in all cases.
2. The cost effectiveness of blood cultures is questionable.
3. The impact of blood cultures on antibiotic prescribing is negligible as physicians seldom alter antimicrobial therapy.

Waterer et al (2001) studied 209 subjects with CAP all of whom had blood cultures performed. The patients were stratified according to the Pneumonia Severity Index (PSI). They concluded that blood cultures were of minimal value in mild to moderate CAP and

should be limited to patients with PSI grade IV or V. In a retrospective analysis of 517 cases of patients hospitalised with CAP from Atlanta, USA, only 6.6 % had positive blood cultures (Chalasani et al, 1995). All the patients had no underlying risk factors such as HIV disease, steroid therapy, chemotherapy or malignancy. The authors concluded that blood cultures may have limited clinical utility but questionable cost-effectiveness. In another retrospective study of 74 patients with CAP and documented pneumococcemia, Waterer et al (1999) concluded that physicians were reluctant to narrow antibiotic therapy adding to treatment cost and reducing the impact of blood cultures on management. Blood cultures altered management in only 41.9 % of patients. Of 51 patients with penicillin sensitive pneumococcus, only 21 % (11) were switched to penicillin therapy. Therefore there is a need to clearly define the utility of blood cultures in CAP, and in particular in HIV infected subjects. Research must look at molecular methods and sampling secretions at sites of infection.

1.9 PCP

1.9.1 History of pneumocystis carinii species hominis

Pneumocystis carinii, previously classified as a protozoa, but reclassified as a fungus, is now known as *Pneumocystis jirovecii* (Thomas et al, 2004.) It was discovered in 1909 by Carlos Chagas when he noted cystic organisms in the lungs of guinea pigs infected with *Trypanosoma cruzi*. In 1910 Antonio Carini made similar observations in rats infected with *Trypanosoma lewisi*. Both scientists mistakenly concluded that these organisms represented a stage in the development of the trypanosome life cycle. In 1912 these cystic organisms were correctly identified and named in honour of Dr Carini by Drs Delanoe and Delanoe (Levine, 1996).

In 1942 the association between *Pneumocystitis jirovecii* and pneumonia in humans was described for the first time by van der Meer and Brug. They had noted an unusual form of interstitial plasma cell pneumonia in premature infants and malnourished children in Central and Eastern Europe. Further descriptions of the disease were published by Vanek, Jirovec and Lukes in Europe, and in 1958 Ivady and Paldy identified pentamidine isethionate as effective therapy. This reduced the mortality due to PCP from 50% to 3.5%. Isolated cases of PCP related to corticosteroid therapy (equivalent to > 20 mg of prednisolone a day), lymphoma and cytotoxic drug therapy were reported prior to the advent of HIV disease. The first cases of PCP in HIV infection were reported in homosexual men from the USA in 1981. Between 1982 and 1987, the incidence of PCP as the AIDS defining index diagnosis was 44% - 74% (Levin, 1996).

Historically, HIV related PCP has been considered to be rare in Africa. This notion is based on few studies from the African continent during the early stages of the HIV epidemic in the 1980s and 1990s (Elvin et al, 1989; McLeod et al, 1989; Abouya et al, 1992). In West African patients, autopsy studies performed on HIV seropositive patients dying of respiratory related illnesses showed PCP in only 9 % of patients (Abouya et al, 1992). In comparison, tuberculosis was found in 40 % of cases. The explanation could be that patients were dying too early from tuberculosis, bacterial pneumonias and other HIV related complications before they reached the stages of advanced disease associated with PCP. However, from observation and unpublished local data, the incidence of PCP has increased dramatically in African patients. This is supported by data from some recent paediatric studies (Nathoo KJ et al, 2001; Ruffini DD et al, 2002; Madhi SA et al, 2002; Chintu C et al, 2002; Ansari NA, et al, 2003).

1.9.2 Biology of *Pneumocystis jirovecii*

Pneumocystis jirovecii is a eukaryote. Mammals are the only known hosts of *Pneumocystis jirovecii* and the variety of host species includes rabbits, ferrets, rats, mice and humans.

The ubiquitously distributed organism can exist in three forms:

1. The trophozoite
2. The cyst
3. The precyst

The organism cannot be grown freely in cell culture and hence what is known of it has been derived from infected human and rat lungs in short term cultures. There are structural differences among *Pneumocystis jirovecii* species that affect the different hosts. Each *Pneumocystis jirovecii* species is capable of causing disease in that host only. In humans, pneumocystosis is caused by the species *Pneumocystis jirovecii* special form hominis (P.j sp f. hominis).

Genetic diversity exists within *Pneumocystis jirovecii*. Two loci, the mtrRNA and ITS loci, have been studied.

1 The mtrRNA locus consists of a 300 base pair region encoding mtrRNA. Several positions can be occupied by a different base pair (Stringer et al, 1996). One study found seven different sequences among 12 samples (Lee et al, 1993) .The changes that were observed were:

1. A C-to –T change at position 85.
2. A G-to –A change at position 288

3. A single base deletion at position 34
4. An A-to -G change at position 181
5. A C-to-T change at position 248
6. A base deletion at position 85
7. A seventh sequence, which is very different from the prototype sequence, is a hybrid of the human and rat pneumocystis sequences.

2. The ITS locus has two sequences, internal transcribed spacers ITS1 and ITS2 in the nuclear gene encoding cytoplasmic rRNA. Lu and colleagues (1994) classified the ITS1 sequences into two and the ITS2 sequences into three types. By looking at the genotypic variations in *Pneumocystis jirovecii hominis* infections, the epidemiology of the disease, the common genotypes, co infection with the different genotypes and the diseases patterns and virulence associated with the different genotypes can be studied.

1.9.3 Diagnosis of PCP

The institution of empiric therapy versus definitive identification of *Pneumocystis jirovecii* in AIDS related PCP is controversial. Response rates of 95 % and 82 % have been shown in patients with typical presentations (Pozniak et al, 1986; Miller et al, 1989). The typical HIV seropositive patient with PCP presents with insidious onset of symptoms, dry cough, fever, dyspnoea on exertion or at rest, tachypnoea with or without cyanosis, clear chest or a few scattered crackles on auscultation and a chest radiograph showing diffuse bilateral interstitial or alveolar infiltrates (Levine, 1996). The expense, time, discomfort and risk of adverse effects of invasive procedures are avoided. However, one study showed that only 57 % of AIDS patients with suspected PCP actually had *Pneumocystis jirovecii* recovered

from BAL or transbronchial biopsies (Luce et al, 1988). Forty three per cent had Kaposi's sarcoma, bacterial pneumonias, bronchitis or a non-specific diagnosis. These patients would have received inappropriate treatment. Not all studies of empiric treatment have shown high response rates. Possible explanations include wrong diagnosis and drug resistance to trimethoprim sulphamethoxazole. This is difficult to assess. Important factors that cannot be ignored include:

1. Drug toxicity
2. Corticosteroid therapy and the risk of progression of bacterial and mycobacterial infections
3. The emotional effects of an uncertain or incorrect diagnosis
4. Patients on prophylaxis have atypical presentations
5. The therapeutic dilemma of ventilating a patient with an uncertain diagnosis

1.9.4 Diagnostic procedures for PCP

Laboratory techniques for the diagnosis of PCP rely on microscopic demonstration of *Pneumocystis jirovecii* in respiratory tract secretions by means of conventional cytochemical procedures or immunocytochemical staining (Broaddus et al, 1985; Bigby et al, 1986; Linder et al, 1989; Kroe et al, 1997; Walzer et al, 2000). Cysts may be recognized with the use of special stains that stain the cyst wall such as Gomori's methenamine silver and toluidine blue O. Identification of more than five cysts is necessary to diagnose PCP. Trophozoites can be stained with Giemsa, Wright-Giemsa and Diff-Quik stains.

The procedure of sputum induction for the diagnosis of PCP was originally described by Bigby et al (1986). Although the technique is simple and relatively non-invasive, it has a

wide range of accuracy with a sensitivity that averages 55%. Study design, the population studied and staining techniques may partly explain the wide range of accuracy.

Bronchoscopy with BAL has a high sensitivity and specificity of more than 90% and is regarded as the gold standard for the diagnosis of PCP. The procedure is at least as sensitive as fiberoptic bronchoscopy with transbronchial biopsy but is considered less invasive. The increased incidence of PCP as a result of the HIV epidemic has brought about an increased need for less invasive diagnostic techniques. Table 1.9 summarises the currently available methods for PCP diagnosis.

Table 1.9 Induced sputum, bronchoalveolar lavage, and surgical biopsy in the diagnosis of PCP (Miller, 1999)

Technique	Ease of procedure	Diagnostic sensitivity (%)	Cost	Notes
Induced sputum	Simple- once technique established	50 - 90	Low	Requires dedicated staff and facility. Risk to staff from expectorated aerosol
Bronchoalveolar lavage	Moderate	90 - > 95	Moderate	Risk of deterioration post procedure. Risk to staff from coughed secretions. Sensitivity may be increased by two lobe lavage.
Surgical biopsy	Complex	> 95	High	Requires staff with surgical expertise

Amplifying *Pneumocystis jirovecii* DNA obtained noninvasively from oropharyngeal samples is an attractive option. This method has been studied by Wakefield et al (1993). DNA amplification of oropharyngeal washes was performed in 31 HIV seropositive

subjects with respiratory illness. In 10 of 18 patients (56 %) with PCP, *Pneumocystis jirovecii* specific DNA was detected by staining with ethidium bromide and none was detected in 13 patients with other respiratory diagnoses. The more sensitive technique of oligoblotting yielded positive results in 14 (78 %) of the 18 patients with PCP. Thus, the relative yield and diagnostic utility of *Pneumocystis jirovecii* DNA amplification in oropharyngeal samples of HIV infected patients with PCP needs to be studied. Potential problems include extraction of nucleic acid from clinical material, cross contamination with the products of previous assays and clinical interpretation of a test result.

1.10 Management of CAP: South African Thoracic Society guidelines

Guideline documents for the management of CAP have been published world wide by regional and national bodies (Cazzola et al, 2001; Feldman, 1998). These include SATS, BTS, ATS and CTS (Niederman et al, 1993; British Thoracic Society, 1993; Mandell et al, 1993; Feldman et al, 1996). The objectives of most guidelines are to:

1. Promote efficient and effective care
2. Improve patient outcomes
3. Rationalize treatment
4. Promote cost-effective treatment

There are differences in the recommendations of the expert committees. Possible explanations include:

1. Geographical differences in aetiology
2. Changes in antimicrobial susceptibility patterns
3. Cost

4. Severity of pneumonia
5. Emergence of new pathogens
6. The changing demography of patients and the HIV epidemic. While there is an increasing elderly population in Europe, Canada and the USA, HIV/AIDS is the biggest challenge confronting humanity today, especially in sub-Saharan Africa.

Differences in the guidelines include the definition of severe pneumonia and which categories of patients should be hospitalised. There is no universally accepted definition as to what should be considered severe pneumonia. These differences are highlighted in tables 1.10 and 1.11.

Table 1.10 Factors indicating the need for hospitalisation of patients with CAP
(adapted from Feldman C, 1998)

Features	ATS guidelines	RSA (SATS) guidelines
1. Age > 60 years	+	+
2. Comorbidity	+	+
3. Physical features predictive of morbidity		
-confusion	+	+
-respiratory rate >30 breaths /min	+	+
-systolic blood pressure <90 mmHg	+	+
-diastolic blood pressure <60 mm Hg	+	+
-temperature >38.3 degrees Celsius	+	+
-extra-thoracic septic complications	+	+
-laboratory data predicting increased mortality		
-white cell count<4 or >30x10 ⁹ /l	+	+
-PaO ₂ <60 mmHg	+	+
-urea > 7mmol/l	+	+
-multilobar consolidation, cavitation or rapidly expanding infiltrates	+	+
-haemoglobin < 9 g/dl; haematocrit < 30%	+	Not considered
-albumin < 30g/l	Not considered	+
-sepsis, end organ dysfunction	+	Not considered
-consideration of socio-economic circumstances	+	+

Table 1.11 Assessment of severity of pneumonia in hospitalised cases with CAP

(adapted from Feldman C, 1998)

Features	ATS	BTS	CTS	SATS
Age > 60 years	0	+	0	+
Underlying comorbidity		+	0	+
Confusion	0	+	0	+
Atrial fibrillation	0	+	0	0
Respiratory rate >30 breaths /min	+	+	+	+
Shock				
systolic BP <90 mmHg	+	0	0	+
diastolic BP < 60 mmHg	+	+	0	+
need for vasopressors for > 4 hours	+	0	0	+
PaO ₂ < 60mmHg	0	+	+	+
PaO ₂ /FiO ₂ ratio <250	+	0	0	0
Sepsis with end organ dysfunction	0	0	+	0
Extra-thoracic septic complications	0	0	+	+
White cell count <4 or > 30x 10 ⁹ /l	0	+	0	+
Albumin <30g/l	0	+	0	+
Bacteraemia	0	+	0	0
Low urine output	+	0	0	0
Multilobar consolidation; cavitation	+	+	+	+
Urea > 7 mmol/l	0	+	0	+
Need for mechanical ventilation	+	0	0	+

0 = parameter was not mentioned

According to the ATS and the BTS, the presence of any one of the above factors denotes severe pneumonia. The South African guidelines, however, consider the presence of two parameters to define severe pneumonia. Parameters which are considered important by some societies are not considered to be so by others.

The dissimilarities in the guidelines for the treatment of severe cases of CAP are partly due to geographical differences in the aetiology of CAP (Table 1.12). In addition, these recommendations were formulated using a combination of evidence based medicine and

consensus. In South Africa for example, the role of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophilla* in the aetiology of CAP has been hotly debated and there is no agreement on the issue (Myburgh et al, 1993; Maartens et al, 1994; Potgieter, 1995; Maartens et al, 1995). Although the SATS guidelines encourage the use of macrolides in severe CAP, justification is needed for this. Hence it is crucial that more studies on aetiology should be encouraged to provide evidence based recommendations.

Table 1.12 Recommendations of the ATS, BTS, CTS and South Africa for the treatment of severe CAP

	Preferred treatment	Alternative Regimen
ATS	macrolide +/- rifampicin	macrolide
	+	+
	3 rd generation cephalosporin with anti-pseudomonas cover	imipenem/cilastatin or ciprofloxacin
BTS	2 nd /3 rd generation cephalosporin	ampicillin
	+	+
	macrolide	flucloxacillin
		+
		macrolide
CTS	macrolide +/- rifampicin	macrolide
	+	+
	3 rd generation cephalosporin with anti-pseudomonas cover	imipenem/ cilastatin
		or
		ciprofloxacin
SATS	2 nd generation cephalosporin	amoxycillin/clavulanate
	+	+
	aminoglycoside	aminoglycoside
	+	+
	macrolide	macrolide

Expert committees from France, Spain, and Italy have published their own recommendations (Table 1.13).

Table 1.13 French, Italian and Spanish recommendations for initial empirical antibiotic treatment of CAP

Country	Non-severe pneumonia	Severe pneumonia
France	amoxillin 1g t.i.d or macrolide	co-amoxiclav +(macrolide or fluoroquinolone) or 3 rd generation cephalosporin+ (macrolide or fluoroquinolone)
Italy	beta-lactam/ beta-lactamase inhibitor+- macrolide	2 nd /3 rd generation cephalosporin +- macrolide
Spain	procaine penicillin 1,200,000 U b.i.d. or erythromycin (ethylsuccinate) 2-4 g day per day.	3 rd generation cephalosporin + erythromycin

Comorbidity, patient age, disease severity and need for hospitalization are factors taken into account in the North American guidelines. Macrolides, followed by tetracyclines, are first line antibiotics for the management of non-severe patients without co-morbid disease while a second generation cephalosporin is recommended for patients with comorbid disease. In severe pneumonia a second or third generation cephalosporin is recommended together with the addition of a macrolide. Patients admitted to ICU are treated with an intravenous macrolide, rifampicin and an anti-pseudomonal antibiotic. Rifampicin is recommended for suspected *Legionella pneumophila*. There may be an additional benefit and relevance in the South African context with its huge burden of pulmonary tuberculosis.

The South African guidelines took into account the high incidence of infection with *Klebsiella pneumoniae* in ICU studies in the country (Feldman, et al; 1989; Feldman et al, 1995). A second generation cephalosporin provides good, balanced cover against common Gram negative organisms such as *Klebsiella pneumoniae* as well as against Gram positive organisms such as pneumococcus. Until the initial microbiology results are known, aminoglycosides are recommended to cover for pseudomonas aeruginosa as well as

klebsiella pneumoniae. There is sufficient data to support this recommendation (Feldman et al, 1989; Feldman et al, 1990). Macrolides are recommended routinely in severe CAP until such time that the epidemiology of atypical bacteria has been clarified.

There is scope for research. It is not known whether the guidelines are appropriate, and whether their implementation reduces the length of hospital stay and mortality. The epidemiology of CAP in sub-Saharan Africa is changing due to HIV/AIDS and the emergence of *Pneumocystis jirovecii* and *Mycobacterium tuberculosis* as common pathogens. Coinfection with multiple pathogens is a further complicating factor. The current SATS guidelines need to be modified to take into account the increasing incidence of PCP and tuberculosis in HIV seropositive subjects. In the evaluation of the guidelines, the SATS definitions of severe CAP should be applied as it would be expected that South African practitioners would be familiar with them.

1.11 An overview of diagnostic techniques in CAP

1.11.1. Sputum examination

Sputum examination is the diagnostic method of choice in patients with CAP. Sputum examination is quick, simple, cheap, and does not require specialized training. In addition, it is noninvasive. Gram stain may guide initial antibiotic therapy.

Ideally, frankly purulent material should be selected for sputum microscopy and culture.

A good quality sputum sample must have 10 or less epithelial cells and 25 or more neutrophils when examined under low power (x100) (Skerrett, 1999; Park et al, 1995;

Donowitz et al, 2000). A sputum sample not fulfilling the above is unsuitable for Gram stain and culture and should not be processed.

The pitfalls and limitations of sputum examination are:

1. Oropharyngeal contamination rendering some samples unsuitable for processing
2. The overall usefulness of sputum examination for the diagnosis of CAP is not clear. The presence of organisms in sputum may be due to colonization. Whether an organism is causing infection and disease, or colonization is considered in the context of host characteristics. Colonization is considered to be present when the presence of an organism in a host does not cause a specific immune response or infection (Osterholm et al, 2000).

The specificity of Gram stain for identifying pneumococci has been shown to be 85 %, with a sensitivity of 62 % when strict criteria for Gram stain positivity are used (predominant flora or more than 10 gram positive, lancet shaped diplococci per oil immersion field or both). *Haemophilus influenzae* appears as a small Gram negative coccobacillus. Gram stain has a sensitivity of 40 % to 80 % for detecting *Haemophilus influenzae* on sputum examination. Overall the sensitivity of Gram stain in detecting organisms that have been isolated from blood culture is in the region of 85 %. The sensitivity of sputum culture is less. Only 50 % to 60 % of sputum cultures are positive in patients with pneumococcal bacteraemia. In HIV seropositive subjects with AIDS and PCP, as many as 50 % have the diagnosis made on expectorated sputum examination using commercially available monoclonal antibodies or Giemsa's, Gomori's methanamine silver, or toluidine blue O stains.

3. Antigen detection by the quelling reaction, counterimmunoelectrophoresis and coagglutination has been used to improve the sensitivity of organism detection in respiratory secretions. In pneumococcus, this may be improved to 70 – 90 %. However

these techniques have not been utilized as expected for several reasons. They include difficulty with deciding on colonization versus infection; cross-reactivity between organisms, and prior antibiotic therapy lowering the overall sensitivity.

The direct fluorescent antibody assay for *Legionella pneumophila* has a specificity of over 90 %. The sensitivity is not as good, ranging from 25 – 75 %. The assay does not detect non-pneumophilla strains of legionella. Enzyme immunoassay detection of *Chlamydia trachomatis* and *Chlamydia pneumoniae* polysaccharide has low sensitivity of 20 – 60 %. Numerous problems make this test unattractive and these include cross reactivity with normal flora, relatively low amounts of lipopolysaccharide in respiratory secretions and the inability to distinguish one chlamydia species from another.

DNA amplification using PCR has been applied to detect *Legionella pneumophila*, *Mycoplasma pneumoniae*, *chlamydia pneumoniae*, and *Cytomegalovirus* and *Pneumocystis jirovecii*. Although PCR has high sensitivity, the technique has remained largely experimental. This is because of the high risk of picking up contaminants due to the high sensitivity; the difficulty in differentiating between infection and colonization and between active, latent and past infection and the occurrence of false negative results due to the presence of natural inhibitors.

4. Identifying an organism on Gram stain does not necessarily imply that it is the cause of the pneumonia. Clinical and microbiological correlation is essential.
5. Not all patients with pneumonia produce sputum. Up to 40 % of patients do not produce sputum.

6. The usefulness of sputum culture in the diagnosis of CAP is not quite clear (Fiala, 1969; Barrett-Connor, 1971; Thorsteinson et al, 1975; Davidson et al, 1976; Drew, 1977; Guzzetta et al, 1983; Gleckman et al, 1988). Forty five to 50 % of patients with pneumococcal bacteraemia have negative sputum cultures despite large numbers of organisms on Gram stain. In patients with positive blood cultures for *Haemophilus influenzae* 34 to 47 % have negative sputum cultures. Other notable problems include lack of correlation between sputum cultures and blood cultures and serologic studies. Thirty two per cent of sputum cultures are contaminated with Gram negative organisms from the oropharynx.

Good sputum quality, rapid processing of samples, quantitative cultures, washing of samples are thus important considerations in minimizing these problems and maximizing the diagnostic utility of sputum cultures. The isolation of *Mycobacterium tuberculosis*, *Legionella species* and *Pneumocystis jirovecii* from sputum is diagnostic regardless of the sputum quality (Donowitz et al, 2000)

1.11.2 Transtracheal aspiration

This is a more direct method of obtaining lower respiratory tract secretions. Although the samples produced are less likely to be contaminated from the oropharynx, the risk of complications has limited its utility as a diagnostic technique (Pecora, 1959; Irwin et al, 1981; Ostergaard et al, 1993; Skerrett, 1999)

1.11.3 Fibreoptic bronchoscopy

Fibreoptic bronchoscopy allows direct access to the lower respiratory tract. Samples can be obtained by way of bronchial washings, lavaging a segment or segments of bronchi and alveoli (BAL) with or without a protected specimen brush. In addition, a transbronchial biopsy can be performed to obtain samples of lung tissue.

The major limitation of fibreoptic bronchoscopy is that the procedure is invasive and is considered only in a selected group of patients who are stable and not responding to empiric antibiotic therapy. The risk of serious complications such as a significant pneumothorax or major bleeding is low, being less than 5 %. The usefulness of the procedure is limited by bacterial contamination from oropharyngeal flora. Although the protected specimen brush might reduce this risk, quantitative culture has been utilized to differentiate contaminants from true infecting agents (Hayes et al, 1980; Fletcher et al, 1983; Wimberly et al, 1982; Skerrett, 1999). Studies have shown that in areas of lung with pneumonia, 10^6 to 10^8 microorganisms/ml are present. Bacterial growth of more than 10^3 to 10^4 microorganisms/ml is considered clinically significant. Specimens obtained by the protected specimen brush have a sensitivity of 70 to 90 % and a specificity of 95 to 100 % for the diagnosis of bacterial pneumonia.

In certain categories of patients, fibreoptic bronchoscopy is not useful. These include patients who have received prior antibiotics, patients who have purulent bronchitis as well as those with structural disease. Specimens obtained bronchoscopically from these patients have yielded significant numbers of organisms even in the absence of pneumonia. The procedure is therefore reserved for suitable candidates with severe pneumonia, unresolving pneumonia, or cases of antibiotic failure. In areas of infected lung versus uninfected lung, bacterial counts may differ fifty fold and in very early cases of pneumonia, bacterial

numbers may not be high enough to be detected as clinically significant on quantitative culture. This may explain the 30 – 40 % false negative rates reported with bronchoscopy in some series.

1.11.4 Bronchoalveolar lavage

Bronchoalveolar lavage, which can sample as many as 100 million alveoli, utilizes a diagnostic threshold of 10 000 colony forming units per millilitre of fluid (Sorensen et al, 1989; Jimenez et al, 1993; Skerrett, 1999). The sensitivity of the procedure in the diagnosis of *Pneumocystis jirovecii* is 89 - 98 %. In cases of pulmonary tuberculosis in which the sputum cultures have been negative, BAL has sensitivity of 85 %. In miliary tuberculosis, BAL fluid culture has 100 % sensitivity.

1.11.5 Blind endotracheal suctioning

In cases of severe pneumonia requiring ventilation, blind endotracheal suctioning with quantitative culturing compares favourably with bronchoscopic procedures. The cut off threshold used for a significant isolate is 100 000 colony forming units per millilitre of aspirate (Donowitz et al, 2000).

1.11.6 Lung biopsy

In almost all cases of acute pneumonia, biopsy procedures are rarely indicated. This may take the form of transbronchial, thoracoscopic or open lung biopsy. Transthoracic needle aspiration for dense consolidation can yield positive cultures in 33 % to 80 % of cases

(Davidson et al, 1976; Barnes et al, 1988; Bella et al, 1993; Ishida et al, 1998; Skerrett, 1999). Transthoracic lung aspiration is not favoured due to the risk of bleeding and pneumothorax. This occurs in as many as 39 % of cases (Donowitz et al, 2000). Thoracoscopic biopsy has yields of up to 90 % but several factors limit its use. These are the invasive nature of the procedure, cost, expertise and experience required. Open lung biopsy is reserved for patients who are immunocompromised, not improving on empiric therapy and in whom other simpler and cheaper diagnostic procedures have been unhelpful. The diagnostic yield is 60 - 100 % (Donowitz et al, 2000).

1.11.7 Pleural fluid examination

The incidence of pleural effusions in patients with CAP ranges from 10 % with *Streptococcus pneumoniae*, 50 to 70 % with Gram negative bacilli and as high a figure as 95 % with group A streptococci (Donowitz et al, 2000). Isolation of an organism from the pleural fluid is specific for that organism causing pneumonia. The advantages of pleural fluid aspiration and analysis are its simplicity, requirement for minimum expertise and its safety.

1.11.8 Serology

Serology tests for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* lack sensitivity, specificity and availability. The tests provide useful data for epidemiological surveillance but are usually not helpful in the initial evaluation of CAP (Skerrett, 1999).

In *Mycoplasma pneumoniae*, the presence of cold agglutinins in a titre > 1:64 has a diagnostic sensitivity of 30 - 60%. The specificity is however poor. IgM antibodies require up to 7 days to reach diagnostic titres and these persist for 2-12 months (Bartlett et al, 1998).

The value of microimmunofluorescence, PCR and culture in the diagnosis of *Chlamydia pneumoniae* is debatable and few laboratories offer any of these tests (Ramirez et al, 1996).

Culture of *Chlamydia pneumoniae* is technically difficult and not widely available.

A positive culture does not necessarily mean infection and although antigen detection in respiratory secretions has been positive in 20 % to 90 % of cases, this is largely confined to research laboratories (Grayston et al, 1993; Kauppinen et al, 1995; Boman et al, 1997).

Most cases of *Chlamydia pneumoniae* are diagnosed serologically using either complement fixation (CF) or microimmunofluorescence (MIF). IgM and IgG antibodies in primary infection may take up to 3 weeks and 8 weeks to appear respectively. Absence of detectable antibodies therefore does not exclude infection with *Chlamydia pneumoniae*.

With complement fixation, a four fold rise in titer or a single titer greater than 1:64 is considered diagnostic. With MIF, a four fold rise in titer, an IgM titer of 1:16 or greater, or an IgG titer of 1:512 or more are considered diagnostic. Polyclonal gammopathy can interfere with results (Grayston et al, 1993; Kauppinen et al, 1995). Serologies are the reference standards for the diagnosis of *Chlamydia pneumoniae*. This is despite these limitations.

Antibodies to *Legionella pneumophila* in acute infection have a positive predictive value of only 15 % (Plouffe et al, 1995).

In summary, there are no good serological tests that can be used to accurately diagnose acute infections due to *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella species* with a high degree of sensitivity and specificity. The detection of increased levels of specific IgM antibody in a single serum sample can be useful in the early diagnosis of pneumonia caused by these pathogens. Although a fourfold rise in antibody titres between paired specimens is more sensitive and specific, it is logistically difficult to perform. The patient has to return for further tests weeks later. There is no impact on the patient's antibiotic treatment. Serologies are useful in retrospective diagnosis and epidemiologic studies. The logistical difficulties of asking well patients to come back for further serological tests are substantial.

CHAPTER 2

Aims, hypotheses and methods

2.1 Aims

1. To determine the epidemiology of CAP in HIV and non-HIV infected subjects
2. To determine the incidence of PCP in HIV infected patients with CAP
3. To determine the diagnostic utility of PCR for *Pneumocystis jirovecii* DNA in oropharyngeal washings of HIV infected subjects with PCP
4. To investigate adherence to the South African Thoracic Society CAP treatment guidelines and their effect on length of hospital stay and mortality
5. To evaluate the utility of the Fine Score in HIV infected subjects with CAP
6. To determine the relationship between markers of disease severity and the yield from blood cultures and the impact of positive blood cultures on antibiotic prescribing
7. To determine the utility of PCT in distinguishing CAP due to bacteria, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii*

2.2 Hypotheses

1. The epidemiology of CAP has been altered by HIV infection.
2. PCP is not rare in HIV infected African patients with CAP.
3. PCR DNA amplification of oropharyngeal washings is useful for the diagnosis of PCP in HIV infected subjects.
4. SATS pneumonia guidelines have had no impact on physician prescribing, length of hospital stay or mortality.
5. The Fine Score applies equally in both HIV infected and non-HIV infected patients.

6. The yield from blood cultures is not influenced by the severity of CAP and blood cultures do not alter antibiotic prescribing.
7. Serum PCT concentrations differ significantly in pneumonia due to common bacteria, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii*.

2.3 METHODS

2.3.1 Ethical approval and patient consent

Ethical permission for the study was obtained from the Ethics Committee of the Nelson R Mandela School of Medicine, University of KwaZulu-Natal. Informed consent was obtained from all subjects (Appendix 1).

2.3.2 Setting and patient selection

The study was performed at King Edward VIII hospital, Durban, South Africa. It is a tertiary teaching hospital of the Nelson R Mandela School of Medicine, University of KwaZulu-Natal. The hospital serves the predominantly Black African residential areas of Umlazi, KwaMashu, Clermont, Chesterville, Inanda, Ntuzuma as well as patients from the more affluent suburbs of Umbilo and Glenwood. It is also a referral hospital for both the surrounding and far away rural hospitals of KwaZulu Natal. In addition, patients come from as far as the African rural areas of the Eastern Cape Province. Thus, all the patients in this study were Black Africans.

Patients presenting with a diagnosis of CAP to the medical wards were enrolled in the study. Patients were recruited between May 2000 and October 2001.

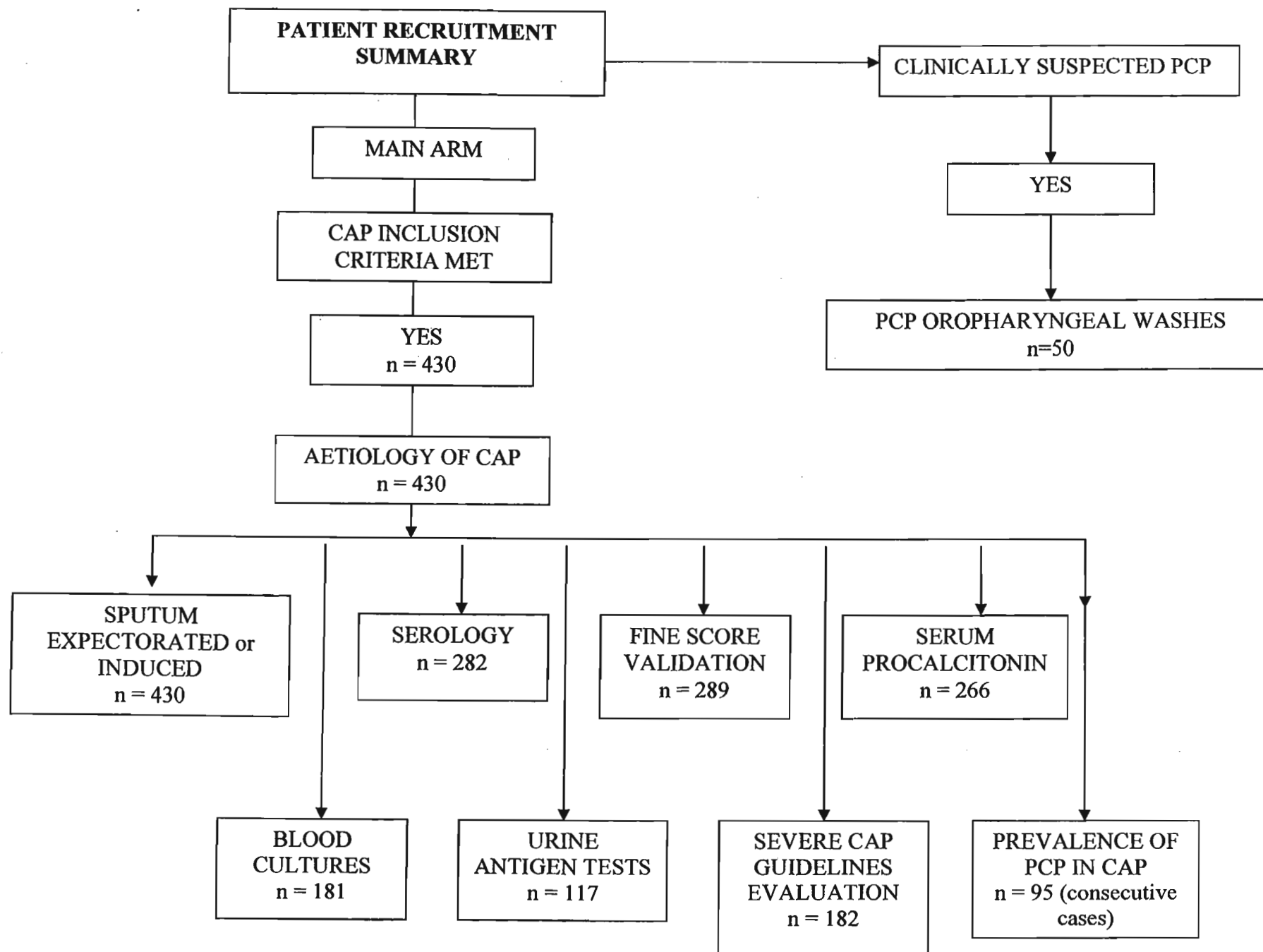


Fig. 2.1 Patient recruitment summary

Definition of CAP

Pneumonia acquired out of hospital.

Criteria for the diagnosis of 'pneumonia'

There are slight variations in the clinical definition of CAP in most studies to date (Mundy et al, 1995; Lieberman et al, 1996; Cordero et al, 2000; Paganin et al, 2004), none of which incorporate the 14 day cut off or define pneumonia in terms of duration of symptoms.

The following criteria were used to define a clinical diagnosis of CAP in this study:

1. The presence of any one of the following symptoms:
 - (a) fever of 37.8 degrees C or more
 - (b) cough with or without sputum production
 - (c) dyspnoea
 - (d) chest pain
 2. Chest radiograph showing the presence of a new infiltrate, opacification or cavitation.
- Both criteria 1 and 2 had to be fulfilled.

Exclusion Criteria

- (a) bronchiectasis
- (b) interstitial lung disease
- (c) pulmonary fibrosis
- (d) pneumonia if known to be associated with bronchial carcinoma
- (e) age < 18 years
- (f) failure to give informed consent

The study of persistent or recurrent pneumonia was not included in the study protocol. The patients with clinically suspected PCP who had oropharyngeal washes were recruited over a 6 month period between March 2000 and September 2000. These were patients with clinically suspected PCP ie dyspnoea, cough, tachypnoea with minimal chest signs on auscultation and bilateral interstitial infiltrates. Cost and the invasive nature of the investigations necessitated this relatively short recruitment period.

2.3.3 Assessment of patients and data collection

Full history and clinical evaluation was undertaken at initial assessment. The following parameters and clinical signs were recorded:

- (a) age
- (b) sex
- (c) duration of symptoms
- (d) marital status
- (e) employment status
- (f) alcohol history
- (g) smoking history
- (h) past medical history
- (i) drug history
- (j) temperature
- (k) pulse rate
- (l) systolic blood pressure
- (m) diastolic blood pressure
- (n) respiratory rate

- (o) temperature
- (p) oral thrush
- (q) lymphadenopathy
- (r) clubbing
- (s) hairy leucoplakia
- (t) melanonychia
- (u) skin rash
- (v) weight loss (ie temporal or general muscle wasting, or a change in clothes size as perceived by the patient)
- (w) chest signs
- (x) hepatosplenomegaly
- (y) chest radiographic abnormalities including pleural effusions and mediastinal lymphadenopathy
- (z) initial antibiotic regimen

2.3.4 Length of stay and mortality

The data was extracted from records in the patients' in-patient charts at discharge from hospital or death. Length of stay was recorded in days. The first day of admission was taken as day 1. Empiric treatment for CAP was started on the first day of admission (day 1).

2.3.5 Sample collection

2.3.5.1 Blood

Whole venous blood was obtained from all patients with CAP by venepuncture and drawn into a vacutest tube containing 0.5 ml of 3.8 % sodium citrate. Blood for serum samples was drawn into plain tubes containing no additives.

Flowcytometry: Immunophenotyping (Lyse technique)

Samples for CD4 counts were drawn by venesection into 5 ml volume tubes (5 ml whole blood, anticoagulant –EDTA). Samples were gently mixed by inversion and transported to the laboratory. The procedure is described in appendix 9

Serum for PCT

Blood samples were drawn and put in ice enroute to the laboratory where they were centrifuged for 5 minutes at 1000g. The serum was stored at -85°C pending analysis.

Blood cultures

The venepuncture site was cleaned with 70 % isopropyl or ethyl alcohol. Tops of blood culture bottles were disinfected with alcohol and allowed to dry. Whole venous blood was obtained by venepuncture and 10 mls immediately inoculated into anaerobic and aerobic blood culture media

Procedure is described in Appendix 10.

2.3.5.2 Urine

Samples were voided into plain urine bottles and stored at -8° C until assayed for *Legionella pneumophila* and *Streptococcus pneumoniae* urinary antigens.

2.3.5.3 Oropharyngeal washings

After an overnight fast, patients were made to gargle 10 mls of normal saline (0.9 %). This was decanted into specimen bottles. There after, 5 mls of sample was aliquotted into tubes and stored at -85° C until assayed.

2.3.5.4 BAL fluid and lung tissue

After an overnight fast, patients were nebulized with salbutamol for 10 minutes. Two mls of robinul and 2 mg of midazolam were then administered intravenously and the vocal cords anaesthetized with a 2 % xylocaine spray. An Olympus fibreoptic bronchoscope was then passed via the oral route and the vocal cords visualized. 2 % liquid marcaine was then instilled into the trachea via the open cords. The trachea, carina and bronchi were inspected. Bronchoalveolar lavage of the most affected lung segments (on chest radiograph) was performed with 50 mls of 0.9 % saline per lavage. A maximum 3 lavages were performed. Transbronchial biopsies were taken from the same segments using a flexible biopsy forceps. Four to six pieces of lung tissue were obtained per patient and suspended in 5 % formal saline (41 % formaldehyde/0.9 % sodium chloride, 1:8 v/v).

BAL fluid samples were aliquotted into tubes and stored at -85° C.

2.3.5.5 Sputum

Expectorated Sputum

Samples were collected in sputum bottles and delivered to the microbiology laboratory for processing by a qualified microbiologist.

Induced Sputum

Patients were instructed to fast over-night. Using a De Velbiss ultrasonic nebulizer, subjects were nebulized with 3 % hypertonic saline for 5 minutes. All expectorated secretions were collected in a clean sputum container for microbiology processing.

2.3.6. Sample storage

Serum samples for procalcitonin, serology, or pharyngeal washings and BAL fluid were stored at -85°C till assayed. Urine samples were stored at -4°C .

2.3.7 Sample processing

2.3.7.1 Blood cultures

Aerobic and anaerobic blood cultures were incubated at 37°C . Procedure is described in appendix 10.

2.3.7.2 Sputum samples

Sputum samples were transported to the laboratory for Gram stain, Ziehl Neelsen stain, fungal staining and *pneumocystis jirovecii* immunofluorescence (See appendix 11, 12 and 13).

2.3.7.3 Urine antigen tests for *Streptococcus pneumoniae* and *Legionella pneumophila*

Streptococcus pneumoniae

Principles of the procedure

The Binax NOW *Streptococcus pneumoniae* urinary antigen test is an immunochromatographic membrane assay used to detect pneumococcal soluble antigen in human urine. Rabbit anti-*streptococcus pneumoniae* antibody, the Sample Line, is adsorbed onto nitrocellulose membrane. Control antibody is adsorbed onto the same membrane as a second stripe. Both rabbit anti-*streptococcus pneumoniae* and anti-species antibodies are conjugated to visualizing particles that are dried onto an inert fibrous support. The resulting conjugate pad and the striped membrane are combined to construct the test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a hinged, book-shaped test device.

A swab is dipped into the urine specimen, removed, and then inserted into the test device. Reagent A, a buffer solution, is added from a dropper bottle. The device is closed, bringing the sample into contact with the test strip. Pneumococcal antigen present in the

urine sample reacts to bind anti-*streptococcus pneumoniae* conjugated antibody. The resulting antigen – conjugate complexes are captured by immobilized anti-*streptococcus pneumoniae* antibody, forming the sample line. Immobilized control antibody captures anti-species conjugate, forming the Control Line. Test results are interpreted by the presence or absence of visually detectable pink to purple coloured lines.

Details of procedure are described in Appendix 7. The test kit was manufactured by Bina, Inc. 217 Read St, Portland, Maine 04103 USA.

Legionella urinary antigen test

The Binax NOW legionella Urinary Antigen Test is an immunochromatographic membrane assay to detect *Legionella pneumophila* serogroup 1 soluble antigen in human urine. Rabbit anti-*legionella pneumophilla* serogroup 1 antibody, the patient line, is adsorbed onto a nitrocellulose membrane. Goat anti-rabbit IgG, the control line, is adsorbed onto the same membrane as a second stripe. Rabbit anti-legionella serogroup 1 antibodies are conjugated to visualizing particles that are dried onto an inert fibrous support. The resulting conjugate pad and the striped membrane are combined to construct the test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a hinged, book-shaped test device.

A swab is dipped into the urine specimen, removed, and then inserted into the test device. Reagent A is added from a dropper bottle. The device is then closed, bringing the sample into contact with the test strip. *Legionella pneumophila* serogroup 1 urinary antigen captured by immobilized anti *Legionella pneumophila* serogroup 1 antibody reacts to bind

conjugated antibody. Immobilized goat anti-rabbit IgG also captures visualizing conjugate, forming the Control line.

The test is interpreted by the presence or absence of visually detectable pink to purple coloured lines.

The assay procedure is described in Appendix 8. The test kit was manufactured by: Binax, Inc. 217 Read St, Portland, Maine 04103 USA.

2.3.7.4 Oropharyngeal samples: DNA extraction and PCR for *Pneumocystis jirovecii*.

DNA was extracted from oropharyngeal washings using a Body Fluid Spin Protocol (appendix 3).

The Siam DNA Mini Kit and Siam DNA Blood Mini Kit Handbook were manufactured and supplied by QIAGEN Worldwide.

The DNA was then stored at -20°C before PCR.

2.3.7.5 Serology for *Mycoplasma pneumoniae*

Principle of the assay

Enzyme-linked immunosorbent assays (ELISA) rely on the ability of biological materials to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if

present, will bind to the antigen on the solid phase forming antigen – antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of substrate and chromogen, Tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue colour develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The colour, which is proportional to the concentration of the antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader. The sensitivity, specificity and reproducibility of enzyme-linked immunoassays can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, haemagglutination and radio immunoassays. The kit and instructions for use of the mycoplasma IgM assay were supplied by Trinity Biotech USA; Jamestown NY 14701. The procedure is described in appendix 4

2.3.7.6 Serology for *Chlamydia pneumoniae*

Principle of the test

The principle of the Thermo Labsystems *Chlamydia pneumoniae* IgM EIA kit is based on an indirect solid-phase enzyme immunoassay with horse-radish peroxidase as a marker enzyme.

1. *Chlamydia pneumoniae* IgM antibodies from the patient sample bind to *Chlamydia pneumoniae* antigen attached to the polystyrene surface of the microstrip wells.
2. Residual patient sample is removed by washing and horseradish peroxidase conjugated anti-human IgM (sheep) is added.

3. Unbound conjugate is washed off and a colourless enzyme substrate (H_2O_2) containing the chromogen (TMB) is added. The enzyme reaction with the chromogen results in a coloured end product.
4. The colour formation reaction is terminated by adding acid (H_2SO_4). The colour intensity is directly proportional to the concentration of *Chlamydia pneumoniae* antibodies in a patient sample.

The kit and instructions for use of the *Chlamydia pneumoniae* IgM EIA were manufactured and supplied by Thermo Labsystems Oy, Sorvaajankatu 15, FIN-00810, Helsinki, Finland.

The assay procedure is described in appendix 5.

2.3.7.7 Measurement of PCT concentrations

Principle of the Assay

LUMI test PCT is an immunoluminometric assay (ILMA) used to quantitate the concentration of PCT in human serum and plasma. Two antigen specific monoclonal antibodies that bind PCT (the antigen) at two different binding sites (the calcitonin and katalcalcine segments) are thereby added in excess. One of these antibodies is luminescence labelled (the tracer), and the other is fixed to the inner walls of the tube (coated tube system).

During the course of incubation, both antibodies react with PCT molecules in the sample to form “sandwich complexes”. As a result the luminescence labelled antibody is bound to the inner surface of the tube. Once the reaction is completed, the excess tracer is completely removed from the tube and discarded.

Then the amount of residual tracer on the test tube wall is quantified by measuring the luminescence signal using a suitable luminometer and the LUMItest Basiskit reagents. The intensity of the luminescence signal (RLU) is directly proportional to the PCT concentration in the sample. After a standard curve has been established using standard with known antigen concentrations, the unknown PCT concentrations in patient serum or plasma samples can then be quantitated by comparison of test values with the curve.

Alternatively, using calibrators instead of standards the unknown PCT concentrations in patient serum or plasma samples can be quantitated by comparison of test values with a master curve made by B.R.A.H.M.S Aktiengesellschaft.

The PCT LUMItest kits and instructions were supplied by B.R.A.H.M.S. Aktiengesellschaft; Neuendorfstrasse 25; 16761 Henningsdorf near Berlin; Germany. The test procedure is described in appendix 6

2.3.7.8 Histology

All transbronchial lung biopsy specimens were processed and analyzed by a qualified histopathologist. In addition to standard Haematoxylin and Eosin stains, a Silver stain (Gomori's methanamine) was also performed on all cases together with Gram, PAS and Ziehl-Neelsen stains. Several sections on each case were examined.

2.3.7.9 PCR for *Pneumocystis jirovecii* DNA

The primers used were based on the gene encoding the large subunit mitochondrial ribosomal RNA (mt LSU rRNA). The oligonucleotide primers consisted of an outer primer pair pAZ102-E and pAZ102-H and an inner primer pair P1 and P2. The primers are shown below:

PAZ102-E:- 5'-GATGGCTGTTTCCAAGCCCA-3'

PAZ102-H:-5'-GTGTACGTTGCAAAGTACTC-3'

P1:-5'-CTAGGATATAGCTGGTTTTTC-3'

P2:-5'-TCGACTATCTAGCTTATCGC-3'

The procedure is described in appendix 14.

2.3.8 Statistical analysis

The two-tailed paired student's t-test and the one way analysis of variance (ANOVA) tests were used to calculate significance. Where applicable, results are presented as the mean and standard deviation of the mean. Correlations were determined using the Pearson's correlation coefficient. Statistical significance was considered if the p value was < 0.05. Details of statistical methods are included in each chapter where applicable.

CHAPTER 3

Aetiology of CAP in a mixed cohort of HIV and non-HIV infected adults

3.1 Summary

Aim of Study

To determine the aetiology of CAP in HIV and non-HIV infected adults admitted to King Edward VIII hospital, Durban.

Design

Patients admitted to the medical wards with a diagnosis of CAP between June 2000 and October 2001 were studied. The study was prospective. Samples of sputum, blood, serum, pleural fluid, urine and bronchoalveolar lavage fluid where applicable were collected for microbiological testing.

Results

A total of 430 patients, mean age 33 years, range 18 - 82 years were enrolled. Three hundred and eleven of the 382 patients tested (81.4 %) were HIV infected. Aetiological agents were isolated in 222 patients (52 %). The commonest organisms were *Mycobacterium tuberculosis* (39.6%) and *Streptococcus pneumoniae* (34.5 %).

Conclusions

Mycobacterium tuberculosis and *Streptococcus pneumoniae* are the leading causes of CAP in this study. Compared to previous studies, HIV infection has altered the epidemiology of CAP. New treatment algorithms need to be developed.

3.2 Introduction

Numerous studies on the aetiology of CAP have been published. Most of the studies were performed in the developed world (Mandell, 1995; Mundy et al, 1995; Bartlett et al, 1998; Ruiz et al, 1999; Sopena et al, 1999; El-Solh et al, 2001). Observed differences in the aetiology of CAP are attributable to the degree of diagnostic testing, the population demographics, seasonal patterns, study duration, study design and more recently the impact of the HIV epidemic.

In sub-Saharan Africa, studies of the aetiology of CAP have been scarce, incomplete, selective and most were performed in the pre-HIV era (Prout et al, 1983; Maartens et al, 1994; Feldman et al, 1995). More studies are required.

3.3 Patients and Methods

Between June 2000 and October 2001, all patients admitted to the adult medical wards with respiratory symptoms of pneumonia, and an abnormal chest radiograph of a new infiltrate or consolidation consistent with pneumonia were prospectively studied. Recruitment was performed over 16 months to cover for seasonality and to recruit a reasonable sample size (though the long duration precluded analysis of seasonality).

Microbiological investigations were conducted as described in Chapter 2. In summary, sputum samples were obtained for Gram stain, culture, Ziehl-Neelsen staining, fungal staining and cultures, legionella immunofluorescence. Samples of blood were collected for aerobic and anaerobic cultures and serology. In cases complicated by pleural effusions, microbiological staining and culture for bacteria, mycobacteria and fungi were performed.

Urine samples were obtained for *Streptococcus pneumoniae* and *Legionella pneumophila* antigen tests. ELISA HIV testing was done after appropriate counseling in those patients whose HIV status was unknown. Due to cost constraints, CD4 counts were performed in 195 consecutive patients. For the same reason, two hundred and eight two patients had serology performed for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* IgM. This was between September 2000 and October 2001 to cover for seasonality. Out of 341 patients recruited during this period, samples from 282 patients were analysed. The remaining 59 were found unsuitable for serology testing. These samples had either been haemolysed during or prior to centrifugation or lost during storage or prior to centrifugation.

3.4 Results

A total of 430 patients comprising 269 females (63 %) and 161 males (47 %) were admitted with CAP over a 16 month period. The patients' ages ranged from 18 to 82 years with a mean of 33 years. The average duration of symptoms was 10 days with a range of 1 - 21 days. Three hundred and eleven patients were HIV seropositive (81.4 % of the total number of patients tested) while 48 patients declined to be tested. The CD4 count was performed in 195 patients. Seventy two percent had CD4 counts less than 200 cells/mm³. The mean CD4 count was 153, with a range of 0 – 1223. The aetiology of the pneumonia was identified in 222 patients (52 %) and 2 patients had bronchial carcinoma. In total 255 pathogens were isolated. The commonest pathogen was *Mycobacterium tuberculosis* (39.6 %) followed by *Streptococcus pneumoniae* (34.5%) and *Staphylococcus aureus* (7.5 %) (Tables 3.1; 3.2 and Figures 3.1; 3.2). Two patients, both HIV infected, had *Cryptococcus*

neoformans pneumonia. Two patients had chicken pox pneumonia. One was HIV seropositive and the other patient had chronic renal failure and was HIV seronegative.

Differences in aetiology between HIV and non-HIV infected

Pseudomonas aeruginosa was more prevalent in the HIV negative group than in the non-HIV infected cohort (10 % vs 1%; 4 cases vs 2 cases; $p=0.01$). No differences were noted in the prevalence of the other pathogens isolated (*M. tuberculosis* 33 % vs 40 %, $p=0.54$; *S. pneumoniae* 15 % vs 24 %, $p=0.37$; *S. aureus* 10 % vs 9%, $p=0.7$; *H. influenzae* 8 % vs 4 %, $p=0.4$; *E. coli* 5 % vs 2 %, $p=0.2$).

Urine antigen tests

At admission, 117 (65 %) of 179 patients were able to provide urine samples for pneumococcus and *Legionella pneumophila* testing. Of these 117, 58 (49.5 %) were positive for *Streptococcus pneumoniae* and 2 (1.7 %) for *Legionella*.

Polymicrobial infections

In the 222 patients in whom an aetiological agent was isolated, 34 (16.2 %) had polymicrobial pneumonia (Table 3.3). Twenty-three of these patients (63.8 %) were HIV seropositive, 2 were HIV seronegative and 9 declined to be tested. The most frequent combination was coinfection with *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* in 11 patients.

Mortality

There were 73 deaths (17 %) in total. Mortality was 15.7 % in the HIV infected group and 26.7 % in the HIV negative ($p=0.043$). Of the 34 patients with polymicrobial pneumonia,

13 died (mortality 38 %). The increased mortality with coinfections was highly significant ($p = 0.001450$; Risk ratio = 2.51).

‘Atypical’ pathogens

Serology for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* was performed in 282 patients. In this subset of 282 patients, the commonest isolates were *Mycobacterium tuberculosis* (77 cases, 34.4 %); *Streptococcus pneumoniae* (58 cases, 25.9 %); *Mycoplasma pneumoniae* (38 cases, 17 %); *Chlamydia pneumoniae* (11 cases, 4.9 %) and *Staphylococcus aureus* (8 cases, 3.6 %). See Table 3.2 and Fig 3.3

Of the 38 patients with *Mycoplasma pneumoniae* infections, 20 (52.6 %) had coinfections (Table 3.4). Seven patients (63.6 %) with *Chlamydia pneumoniae* infections had polymicrobial pneumonia (Table 3.5).

HIV infection and atypical pathogens

Eight of the 11 patients (72.7 %) with *Chlamydia pneumoniae* infection were HIV seropositive. Two had negative serology and one had declined to have the test done. Twenty- nine of the 38 cases (76.3 %) of *Mycoplasma pneumoniae* infection were HIV infected, seven were negative and 2 declined.

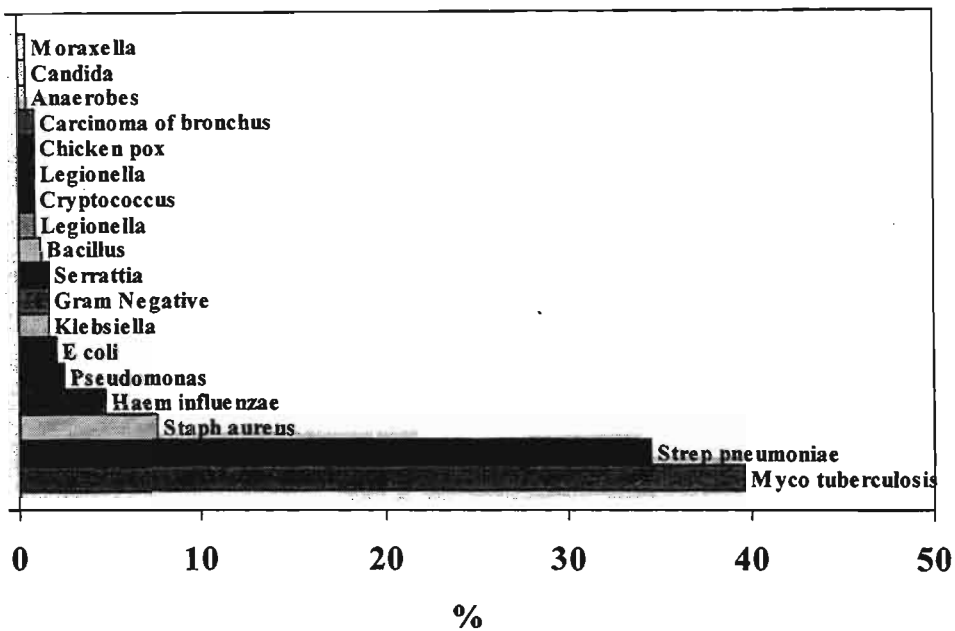


Figure 3.1 Aetiology of CAP in 222 patients. Isolates =255

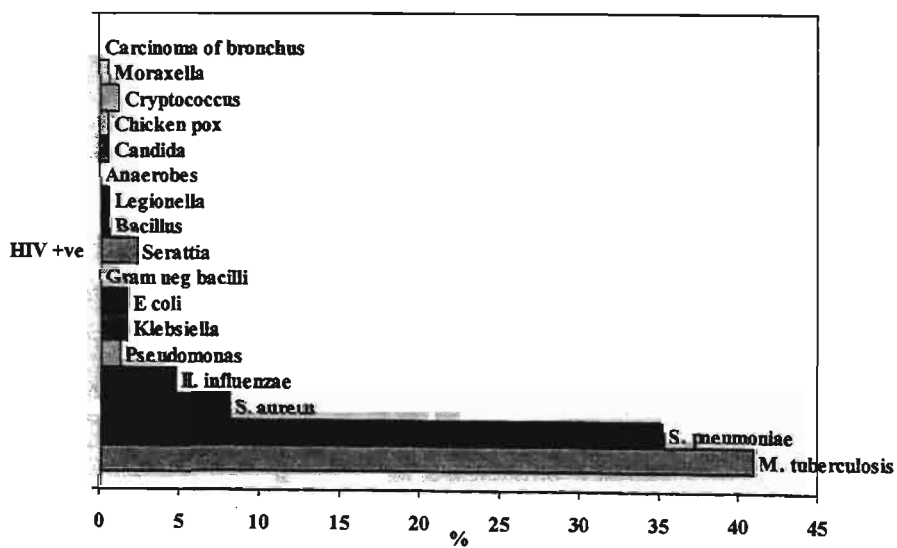


Figure 3.2 Aetiology of CAP in the HIV infected cohort

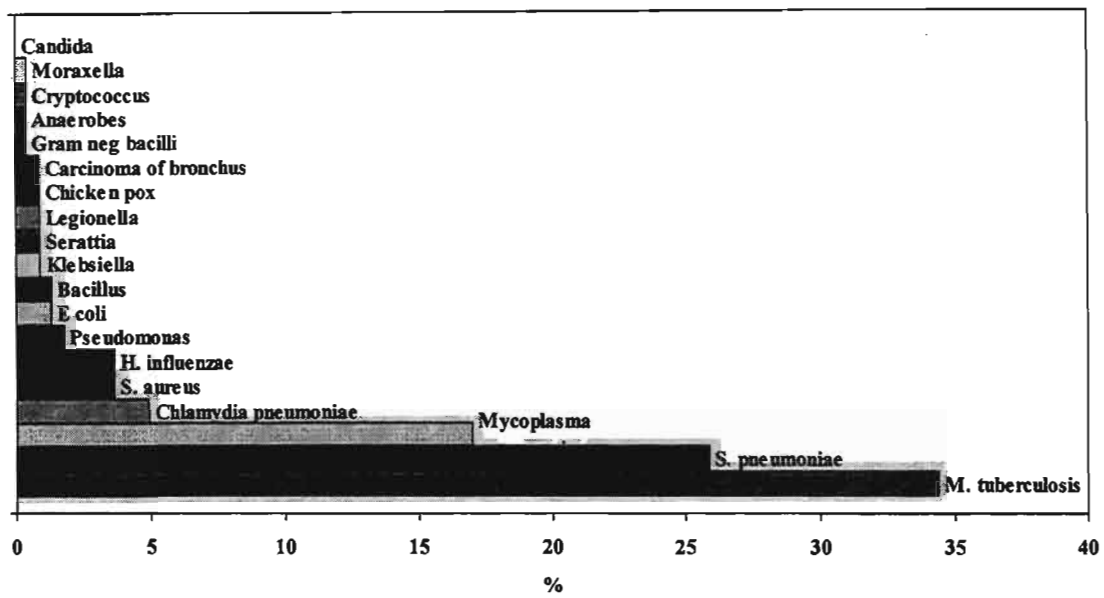


Figure 3.3 Aetiology of CAP in 282 patients who had serology for atypical organisms

Table 3.1 Aetiology of CAP in HIV and non HIV infected subjects

Total Distribution of Organisms					
	HIV+	HIV-	Unknown status	Total number	%
<i>Mycobacterium tuberculosis</i>	71	18	12	101	39.6
<i>Streptococcus pneumoniae</i>	61	12	15	88	34.5
<i>Staphylococcus aureus</i>	14	4	1	19	7.5
<i>Haemophilus influenzae</i>	8	4	0	12	4.7
<i>Pseudomonas aeruginosa</i>	2	4	0	6	2.4
<i>Escherichia coli</i>	3	2	0	5	2
<i>Klebsiella pneumoniae</i>	3	1	0	4	1.6
Gram negative bacilli	3	0	1	4	1.6
<i>Serratia marcesens</i>	4	0	0	4	1.6
Bacillus species	1	2	0	3	1.2
<i>Legionella pneumophilla</i>	1	0	1	2	0.8
<i>Cryptococcus neoformans</i>	2	0	0	2	0.8
Chicken pox	1	1	0	2	0.8
Carcinoma of bronchus	0	2	0	2	0.8
Anaerobes	0	1	0	1	0.4
<i>Candida albicans</i>	1	0	0	1	0.4
<i>Moraxella catarhallis</i>	1	0	0	1	0.4
Total				257	

N=430

Patients with atypical organisms not included

Table 3.2 Aetiology of CAP in 282 patients who had serology for atypical organisms

Aetiology of CAP in 282 patients who had serology for atypical organisms.		
	Numbers	%
<i>Mycobacterium tuberculosis</i>	77	34.4
<i>Streptococcus pneumoniae</i>	58	25.9
<i>Mycoplasma pneumoniae</i>	38	17
<i>Chlamydia pneumoniae</i>	11	4.9
<i>Staphylococcus aureus</i>	8	3.6
<i>Haemophilus influenzae</i>	8	3.6
<i>Pseudomonas aeruginosa</i>	4	1.8
<i>Escherichia coli</i>	3	1.3
Bacillus species	3	1.3
<i>Klebsiella pneumoniae</i>	2	0.9
<i>Serratia marcesens</i>	2	0.9
<i>Legionella pneumophilla</i>	2	0.9
Chicken pox	2	0.9
Carcinoma of bronchus	2	0.9
Gram negative bacilli	1	0.4
Anaerobes	1	0.4
<i>Cryptococcus neoformans</i>	1	0.4
<i>Moraxella cattarhallis</i>	1	0.4
<i>Candida albicans</i>	0	0
Total	224	100%

N=282. The number 224 refers to the total number of isolates

Table 3.3 Coinfections in 34 patients with CAP

Organisms	Number of patients	% of cases
<i>Mycobacterium tuberculosis</i> and <i>streptococcus pneumoniae</i>	11	32
<i>Staphylococcus aureus</i> and <i>haemophilus influenzae</i>	2	6
<i>Mycobacterium tuberculosis</i> and <i>haemophilus influenzae</i>	2	6
<i>Mycobacterium tuberculosis</i> , <i>streptococcus pneumoniae</i> and <i>staphylococcus aureus</i>	2	6
<i>Mycobacterium tuberculosis</i> and <i>staphylococcus aureus</i>	2	6
<i>Mycobacterium tuberculosis</i> and <i>serratia marcesens</i>	2	6
<i>Streptococcus pneumoniae</i> and <i>haemophilus influenzae</i>	2	6
<i>Mycobacterium tuberculosis</i> and <i>klebsiella pneumoniae</i>	1	3
<i>Mycobacterium tuberculosis</i> and <i>cryptococcus neoformans</i>	1	3
<i>Streptococcus pneumoniae</i> and <i>pseudomonas aeruginosa</i>	1	3
<i>Streptococcus pneumoniae</i> and <i>escherichia coli</i>	1	3
<i>Streptococcus pneumoniae</i> and <i>bacillus species</i>	1	3
<i>Streptococcus pneumoniae</i> and Gram negative bacilli	1	3
<i>Streptococcus pneumoniae</i> and <i>staphylococcus aureus</i>	1	3
<i>Streptococcus pneumoniae</i> and <i>haemophilus influenzae</i>	1	3
<i>Escherichia coli</i> and <i>pseudomonas aeruginosa</i> and anaerobes	1	3
<i>Staphylococcus aureus</i> and <i>serratia marcesens</i>	1	3
<i>Klebsiella pneumoniae</i> and <i>pseudomonas aeruginosa</i>	1	3

Table 3.4 Co-infection in 20 patients with *mycoplasma pneumoniae*

Organisms	Number
<i>Mycobacterium tuberculosis</i>	11
<i>Mycobacterium tuberculosis</i> and <i>chlamydia pneumoniae</i>	2
<i>Streptococcus pneumoniae</i>	1
<i>Streptococcus pneumoniae</i> and <i>mycobacterium tuberculosis</i>	1
<i>Streptococcus pneumoniae</i> , <i>haemophilus influenzae</i> and <i>moraxella catarrhalis</i>	1
<i>Haemophilus influenzae</i>	1
<i>Cryptococcus neoformans</i>	1
<i>Chlamydia pneumoniae</i> and <i>bacillus species</i>	1
<i>Streptococcus pneumoniae</i> and <i>bacillus species</i>	1

Table 3.5 Co-infection in 7 patients with *chlamydia pneumoniae*

Organisms	Number
<i>Mycobacterium tuberculosis</i>	3
<i>Bacillus species</i>	2
<i>Mycobacterium tuberculosis</i> and <i>staphylococcus aureus</i>	1
<i>Pseudomonas aeruginosa</i>	1

3.5 Discussion

Mycobacterium tuberculosis was the commonest cause of CAP in the 430 patients studied. It comprised 39.6 % of isolates. In the non-HIV infected and unknown sero status groups, 35 % and 41 % of the isolates were positive for *Mycobacterium tuberculosis* respectively. The presentation of *Mycobacterium tuberculosis*, traditionally a cause of chronic pneumonia, as acute CAP and in such a high proportion, is cause for concern. Treatment guidelines have not taken this into consideration. Most studies of the aetiology of CAP have excluded patients who are HIV seropositive or those with *Mycobacterium tuberculosis* as the aetiology (Sopena et al, 1999; Ruiz et al, 1999; Lim et al, 2001). In a study of 81 patients with CAP from South Africa, *Mycobacterium tuberculosis* was identified in only 6 % of cases while bacterial infections accounted for 37 % (Prout et al,

1983). Leroy et al (1995) studied 299 consecutive cases of pneumonia admitted to intensive care in France. Only one patient was found to have tuberculosis. One large study of 481 patients with CAP found the proportion of patients with *Mycobacterium tuberculosis* to be 2.4 %, 2.2 % and 2.3 % in HIV seronegative, HIV infected and in the whole group respectively (Mundy et al, 1995). From the United Kingdom, Miller et al (1994) found only one case of *Mycobacterium tuberculosis* in 49 episodes of CAP in an HIV infected cohort.

The strong association between *Mycobacterium tuberculosis* and HIV infection in sub-Saharan Africa is well documented (Narain et al, 1992; Ravigilione et al, 1992; Raviglione et al, 1995; Johnson et al, 1998; Antonucci et al, 2001). In 1997, WHO estimated that of the 15.3 million people coinfectd with tuberculosis and HIV world wide, 75 % were in sub-Saharan Africa. The tuberculosis epidemic mirrors the HIV pandemic in the region. The high background tuberculosis prevalence in sub-Saharan Africa contributed to the proportionately high incidence of tuberculosis in this study. As a result, even patients who are HIV seronegative are also at an increased risk of acquiring pulmonary tuberculosis compared to data from the preHIV era. The potentially infective pool, consisting of confirmed and unconfirmed asymptomatic patients with pulmonary tuberculosis, is large. Health care facilities are inadequate and failing to cope. In many areas there is no contact tracing.

Although *Streptococcus pneumoniae* was second to *Mycobacterium tuberculosis* as a cause of CAP in this series, it was the commonest bacterial cause of CAP. This is similar to published data from other parts of the world (Bartlett et al, 1998; Ruiz et al, 1999; Mandell, 1995; Vergis et al, 1999). In HIV infected patients, a number of studies have

found *Streptococcus pneumoniae* to be the commonest bacterial cause of CAP (Miller et al, 1994; Noskin et al, 1996). However, one large study from the USA concluded that *Streptococcus pneumoniae* is surpassed by *Pneumocystis jirovecii* in the HIV-infected population (Mundy et al, 1995).

In the cohort of 282 patients in whom serology for the so called “atypical” organisms was performed, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were ranked third and fourth (17 % and 4.9 % of isolates respectively). Only 2 cases of *Legionella pneumophila* were isolated (0.9 %). In North America and Canada, these organisms are common causes of CAP (Mandell et al, 1995; Bartlett et al, 1998).

The role of atypical pathogens in CAP in South Africa is still a topic for debate (Prout et al, 1983; Feldman et al, 1989; Maartens et al, 1994; Feldman et al, 1995; Maartens, 1997). This study seems to suggest that *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* may be common causes of CAP. However, serological studies for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have low sensitivity and specificity (Skerett, 1999) and absence does not exclude infection (Plouffe et al, 1995; Ramirez et al, 1996). Gold standards of diagnosis have not been established (Vergis et al, 1999).

IgM antibodies were measured in this study. Paired serum to detect four – fold increases in antibody titres are performed only if IgG antibodies are measured. Although the latter may be a better method than the former, the sensitivity is still low. In *Mycoplasma pneumoniae*, four-fold increases in antibody level are seen in approximately 53 % of culture positive patients (Kenny et al, 1990). The incidence of *Mycoplasma pneumoniae* IgG antibodies in

the general population is high while IgM antibodies are detected in acute or recent infection only (Jacobs et al, 1986).

Chlamydia pneumoniae causes approximately 10 – 20 % of CAP in adults and children in Western Europe and the USA (Kleemola et al, 1988, Grayston, 1989; Grayston et al, 1993). Positive IgM antibodies indicate acute *Chlamydia pneumoniae* infection. The method has its limitations. In very early infection IgM class antibodies may not be detectable. In reinfections, IgM may be absent (Grayston et al, 1990; Persson et al, 2000). However, the Thermo Labsystems EIA kit that was used has sensitivity and specificity of 96 % and 99 % respectively (Persson et al, 2000). These results for atypical pathogens are therefore reliable.

Only 2 cases of *Legionella pneumophila* were isolated. This is in agreement with a recent epidemiological study from a tertiary centre in Pretoria, South Africa, that found the incidence to be 1.7 % (Mpe et al, 2001).

An unusual finding in this study was the low incidence of *Haemophilus influenzae* (4.5 %). *Staphylococcus aureus* (7.9 %) ranked higher than *Haemophilus influenzae*. Most studies of the aetiology of CAP rank *Haemophilus influenzae* second to *Streptococcus pneumoniae*. Whether routine vaccination against *Haemophilus influenzae* in childhood would impact on prevalence is not known. Uptake of *Haemophilus influenzae* type b vaccines is poor in the immunocompromised, averaging 40 % as compared to 90 % in HIV seronegative subjects (Miller et al, 1999). More studies are required looking at impact of vaccination.

Cryptococcus pneumoniae should be considered in the differential diagnosis of CAP in the HIV infected population. In this study, one patient was diagnosed from sputum and blood cultures and the second from sputum only.

Coinfections occurred in 16 % of patients. This is a high figure compared to what has been reported. Coinfections have been identified in 2 % - 5 % of patients with CAP (Marrie et al, 1989; Fang et al, 1990; The British Thoracic Society, 1993; Mundy et al, 1995; Keller et al, 1995; Marston et al, 1997). The high figure impacts on treatment protocols. This is particularly important in view of the high mortality found in patients with coinfections in this study. Patients with coinfections were two and half times more likely to die than patients without.

Patients with CAP of undetermined aetiology may have received prior antibiotics that masked the identity of the pathogen. This would have involved the costly analysis of blood or urine samples taken prior to the administering of antibiotics in hospital. Due to the unreliable nature of patients' history regarding prior antibiotic use, this information was not included in the study protocol. Secondly, infection with a pathogen that is as yet to be identified is a remote possibility.

A significant weakness in the study design was the non-performance of diagnostic tests for viral pathogens and for PCP. Two patients had a diagnosis of chicken pox pneumonia based on the classical rash and a bilateral interstitial infiltrate. Both patients were treated with intravenous acyclovir with good response. Respiratory viral infection is diagnosed by tissue culture of nasopharyngeal swabs or nasal wash specimens (Leland et al, 1995). Viral cytopathic effects can be observed in 2 to 14 days after incubation. Since the specimens are

upper respiratory, this does not necessarily equate to the presence of a viral pneumonia (Skerrett, 1999). The definitive confirmatory test for viral pneumonia is demonstration of viral antigens or characteristic inclusion bodies in BAL specimens. DNA amplification of BAL fluid may be utilized (Morris et al, 1996; Yuen et al; 1998). Thus significant financial, material and technical resources are required to achieve complete diagnostic testing for viral pathogens in CAP. This may be difficult to justify as most viral lower respiratory infections are self limiting. The prevalence of PCP was determined in a separate group of consecutive patients (Chapter 4).. It would have been desirable to perform investigations for PCP in all 430 patients. This was not possible for reasons of cost, logistics and human resources.

Mortality was higher in the HIV seronegative cohort (26.7 % vs 15.7 %). This is unexpected. Possible explanations include the small sample size or a differential survival benefit in the HIV group in the short term. The factors for the differential survival may include the use of antibiotics with more broad spectrum cover as well as multiple antibiotics once a patient's HIV status is known. In addition, there is a low threshold to administer trimethoprim-sulphamethoxazole in sick patients with CAP if the HIV serology comes back positive (to cover for PCP). This may have positive survival benefits (broad spectrum antibiotic given in high doses). These data are for short term in hospital mortality.

In summary, *Mycobacterium tuberculosis* was the commonest cause of CAP in both HIV and non-HIV infected patients. *Streptococcus pneumoniae* was the second commonest cause. However, the organism remains the commonest bacterial cause of CAP followed by *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Coinfections are common.

CHAPTER 4

The prevalence of pneumocystis pneumonia

4.1 Summary

Aim of study

To determine the proportion of patients with PCP in a cohort of consecutive cases of CAP

Methods

Ninety-five consecutive cases of CAP were studied. All patients had sputum induction and or fibreoptic bronchoscopy. *Pneumocystis jirovecii* was diagnosed on immunofluorescence of induced sputum, BAL fluid or histology of transbronchial biopsies.

Results

Nine patients (11%) were positive for *Pneumocystis jirovecii*.

Conclusions

Pneumocystis jirovecii is not uncommon in African patients with HIV infection. The organism should be considered an important pathogen in HIV infected patients with CAP.

4.2 Introduction

Pneumocystis pneumonia in Africa was reported as rare in early publications of the pulmonary complications of HIV infection (Elvin et al, 1989; McLeod et al 1989; Carme et al, 1991; Abouya et al, 1992). Grant et al (1998) reported a zero percent incidence of PCP while Koulla-Shiro et al (1996) justified not performing diagnostic tests to detect the organism because of the rarity of the disease.

4.3 Patients and methods

Ninety-five consecutive patients admitted with CAP were recruited between June and August 2000. Diagnostic tests and procedures for PCP prevalence were performed in these 95 only (due to reasons of cost, logistics and technical expertise).

At admission, patients had sputum induction with 3 % hypertonic saline. Bronchoscopy and BAL were performed, with consent, in patients who failed to provide an induced sputum sample after hypertonic saline inhalation. The PCP immunofluorescence test was performed to detect *Pneumocystis jirovecii* organisms in induced sputum. In addition, blood cultures, Gram and Ziehl Neelsen stains and cultures were done on the induced as well as expectorated sputum. Flow cytometry was used to determine the CD4 count. Detailed descriptions of the methods appear in Chapter 2.

4.4 Results

Ninety five patients were recruited into the study. There were 32 males and 63 females and the mean age was 32 years. Eighty-one patients (85 %) were HIV infected. Eighty three patients had sputum induction and 12 patients (13 %) had bronchoscopy. Bronchoscopy was performed in patients who had failed sputum sputum induction. All 12 patients tolerated the procedure well and there were no complications.

PCP was diagnosed in 9 out of the 81 (11 %) HIV infected patients with CAP. There were 7 females and 2 males. The mean age was 33 years with a range of 20 – 56 years. The CD4 count ranged from 0 to 256 with an average count of 91. One patient died (mortality 11 %). There were no cases of PCP in the non HIV infected patients. In 6 patients (67

%), the diagnosis was made from induced sputum and in 3 patients (33 %) from BAL fluid. In the three patients in who PCP was diagnosed from BAL, the histology was negative, showing non-specific chronic inflammation.

There were a total of 81 isolates of 12 different organisms. The commonest organism isolated was *Mycobacterium tuberculosis* (33 subjects), followed by *Mycoplasma pneumoniae* (14), *Staphylococcus aureus* (8) and *Streptococcus pneumoniae* (6). See Table 4.1 and Fig 4.1

When BAL was compared to induced sputum and blood cultures in the 12 patients who had the procedure, the same organism was isolated in 3/16 isolates (19 %) and 2/11 isolates (18 %) respectively. Thus concordance between BAL and induced sputum and BAL with blood cultures was low. Six of the 9 patients with PCP had dual infections (67 %). Two patients had PCP and *Escherichia coli*, two had PCP and *Mycobacterium tuberculosis*, one patient had PCP and *Streptococcus pneumoniae* and one had PCP and *Staphylococcus aureus*.

Table 4.1 Number and % isolates in 95 consecutive patients with CAP

Organism	Number	% Isolates
<i>Mycobacterium tuberculosis</i>	33	41
<i>Mycoplasma pneumoniae</i>	14	17
<i>Pneumocystis carinii</i>	9	11
<i>Staphylococcus aureus</i>	8	10
<i>Streptococcus pneumoniae</i>	6	7
<i>Haemophilus influenzae</i>	3	4
<i>Pseudomonas aeruginosa</i>	2	3
<i>Escherichia Coli</i>	2	3
<i>Klebsiella pneumoniae</i>	1	1
<i>Moraxella catarrhalis</i>	1	1
Gram negative bacillus	1	1
<i>Chlamydia pneumoniae</i>	1	1
<i>Legionella pneumophilla</i>	0	0

Three of the 9 patients with PCP were treated with high dose trimethoprim - sulphamethaxazole based on the clinical presentation. In the remaining 6 cases, PCP was not suspected clinically and the patients were initially treated as bacterial pneumonia.

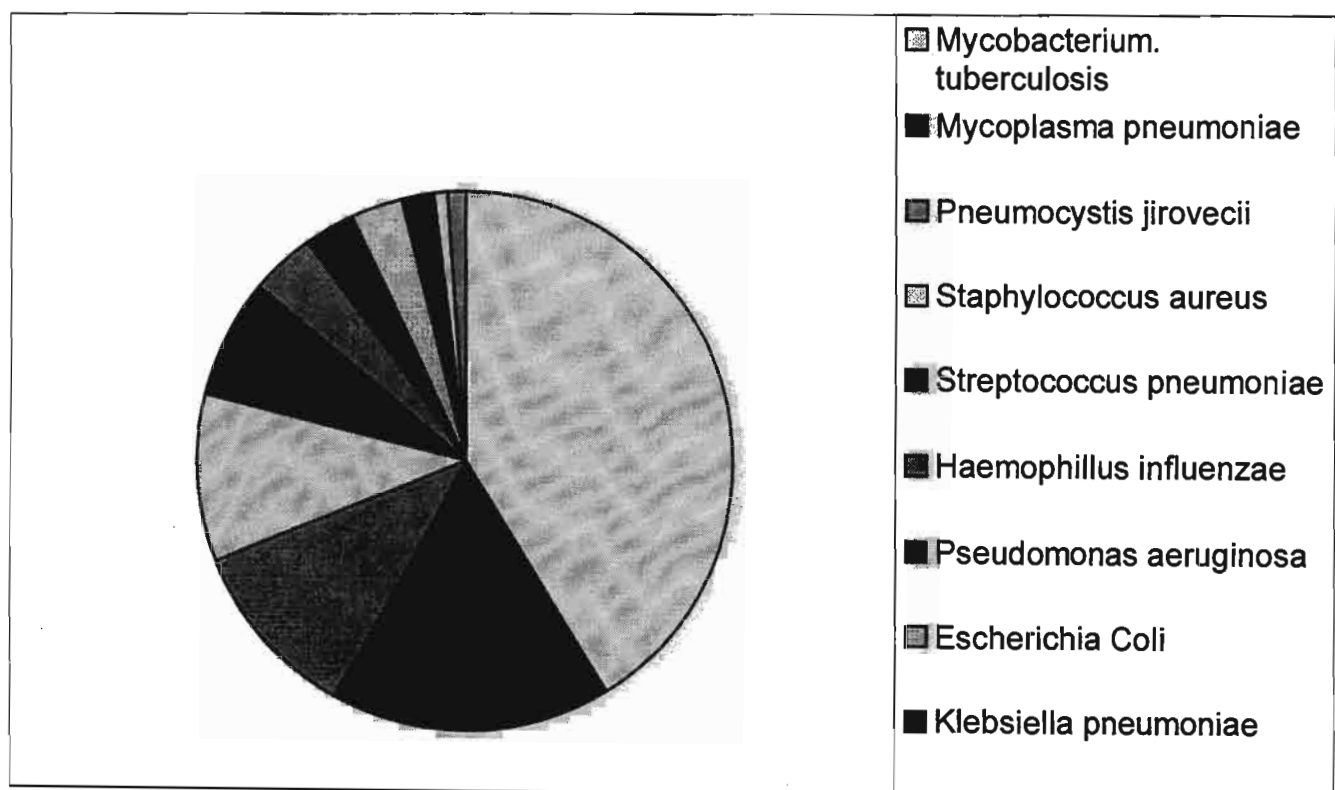


Fig 4.1 % Isolates in 95 patients with CAP

4.5 Discussion

Pneumocystis pneumonia is the commonest opportunistic infection in HIV infected subjects in North America (Levine, 1996). In African patients with HIV infection, there is insufficient data on the incidence of PCP.

In a study of pulmonary diseases in patients infected with HIV from Zimbabwe, *Pneumocystis jirovecii* was found in 8 of 37 patients with respiratory symptoms referred for bronchoscopy over an 11 month period (McLeod et al, 1989). In a study of 78 autopsies of HIV positive patients from West Africa who had died in a pulmonary medicine ward, PCP was found in 9 % (Abouya et al, 1992). Carme et al (1991) from Brazaville, Congo, reported a frequency of 11 % in 45 HIV infected patients with pulmonary infiltrates and negative sputum examination. A retrospective review of 67 HIV infected patients whose sputa were negative for tuberculosis and PCP and who had fiberoptic bronchoscopic evaluation for pulmonary disease between January 1985 and August 2002 in Johannesburg showed PCP as the most frequent disease in patients of both African and European origin. However, it occurred less commonly in Africans than in Europeans (27.3 % vs 58.8 % respectively (Mahomed et al, 1999). The authors' conclusion was that PCP should be considered in the diagnosis of HIV infected African patients with pulmonary disease whose sputum smears for tuberculosis are negative.

This study has shown an incidence of 11 % in the frequency of PCP in a cohort of HIV infected patients presenting with CAP. None of the HIV seronegative subjects had PCP. The studies cited in this chapter focused on a select group of patients referred for further evaluation of pulmonary infiltrates of undetermined aetiology. *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* are the leading causes of pulmonary infiltrates in such patients. This study has demonstrated that even in African patients, PCP should be considered regardless of whether the presentation is typical or not. Only three of the 9 patients had clinical and radiological features suggestive of PCP.

The reason for selectively looking at 95 consecutive patients was due to financial, logistical and technical reasons. A larger sample size would have been desirable but the cost and logistics of performing sputum induction or bronchoscopies on a larger number of patients was not possible under the circumstances. Thus tests for PCP were performed only in these 95 patients (besides the study on PCR and oropharyngeal washes).

Six of the 9 patients with PCP had coinfections. This calls for greater vigilance in HIV sero positive patients with CAP who worsen on appropriate treatment or who do not demonstrate the expected degree of resolution of symptoms. Coinfection with other pathogens should be actively excluded in the appropriate clinical circumstances.

The mortality from a single episode of PCP is about 10 %. Mortality ranges from 12 - 16 % in large series and may be influenced by the number of previous episodes of PCP (Haverkos et al, 1984; Small et al, 1985; Kales et al, 1987; Dohn et al, 1992). The mortality in this study was 11 % and all infections were first episodes. The patient who died was initially treated with intravenous amoxycillin – clavulanic acid for a presumed bacterial CAP on clinical grounds.

The mean CD4 count of 91 is consistent with published studies (Masur et al, 1989; Phair et al, 1990; Levine, 1996). The likelihood of PCP is determined by the degree of immunosuppression as predicted by circulating lymphocyte counts less than 200 cells/mm³. The relative risk is 4.9 in patients with CD4 counts less than 200 cells/mm³. One of the patients with PCP in this study had a CD4 count of 256 cells per mm³. The rest of the patients had CD4 counts less than 200 cells/mm³.

In conclusion, PCP is not uncommon in African patients with CAP and HIV/AIDS. One in ten HIV infected African patients with CAP may have PCP. Therefore a high index of suspicion is necessary as these patients may not have the classical clinical and radiological features of PCP. Coinfections are common in African patients with PCP.

CHAPTER 5

Evaluation of PCR in oropharyngeal washings for the diagnosis of pneumocystis pneumonia in HIV infected patients

5.1 Summary

Aim of study

To compare the relative yield and diagnostic utility of PCR for *Pneumocystis jirovecii* DNA in oro-pharyngeal washings, using transbronchial lung biopsy and bronchoalveolar lavage as gold standards.

Patients and Methods

Oropharyngeal washes were obtained in 50 consecutive patients with suspected *Pneumocystis jirovecii* pneumonia. Because of varying clinical severity not all patients tolerated bronchoscopy. Thirty five patients had transbronchial lung biopsies performed and 48 patients had bronchoalveolar lavage. DNA extracted from oropharyngeal washings and BAL were subjected to a nested PCR test using primers for the large subunit mitochondrial ribosomal RNA of *Pneumocystis jirovecii* and compared to histology of transbronchial biopsies and BAL.

Results

Sixteen of the 35 subjects (46 %) who had transbronchial biopsies had positive histology for PCP. Oropharyngeal washings yielded positive results for pneumocystis in 7 of the 16 patients (sensitivity 44 %, specificity 79 %). Thirty-five of 48 patients (73 %) had positive PCR on BAL fluid analysis. The relative yield of the PCR in oropharyngeal washes compared to bronchoalveolar lavage fluid was 40 % (14 out of 35) giving a sensitivity of 40 % and specificity of 70 %.

Conclusion

DNA amplification of oropharyngeal washings by PCR in HIV infected subjects has a low sensitivity for the diagnosis of *Pneumocystis jirovecii* pneumonia.

5.2 INTRODUCTION

Pneumocystis pneumonia is the most frequent opportunistic lung infection in AIDS (Pitchenik et al, 1986; McLeod et al, 1989; Levine, 1996; Albert et al 1999) with a mortality of 10 - 30 %. The name of the fungus which causes PCP has been changed from *Pneumocystis carinii* to *Pneumocystis jirovecii* (Thomas et al, 2004). Before the era of highly active antiretroviral therapy, up to 75 % (Levine, 1996) of patients infected with HIV developed PCP. Although infection with pneumocystis was initially reported as rare in sub-Saharan Africans with AIDS (Elvin et al, 1989; McLeod et al, 1989; Abouya et al, 1992), the incidence seems to be on the increase as the HIV pandemic progresses and patients with advanced disease present to hospitals. Despite notable advances in the provision of antiretroviral treatment, PCP remains a significant cause of morbidity and mortality in those with limited access to health care and who cannot afford expensive specific antiretroviral therapy or chemoprophylaxis.

Transbronchial or open lung biopsy, fiberoptic bronchoscopy with bronchoalveolar lavage and sputum induction have been the three established methods by which samples are obtained for the diagnosis of PCP. The former two are invasive and carry the risk of significant morbidity and mortality. All three procedures are contraindicated in seriously ill patients.

The aetiology of pulmonary infiltrates in HIV is a major diagnostic challenge (Levine, 1996; Albert et al, 1999). It is important to distinguish between PCP, bacterial pneumonia and pulmonary tuberculosis, particularly in sub-Saharan countries which have experienced a phenomenal increase in HIV associated tuberculosis (Raviglione et al, 1995). In addition, polymicrobial pulmonary infections are well recognized in HIV. An erroneous or missed diagnosis has important implications in terms of cost, drug side effects, morbidity and mortality.

5.3 PATIENTS AND METHODS

Details on methods and patient population have been described in Chapter 2.

HIV sero-positive subjects admitted to the medical wards from March to September 2000 presenting with respiratory symptoms together with pulmonary infiltrates on chest radiographs suggestive of PCP, were studied prospectively. PCP was suspected on the basis of a dry or minimally productive cough, dyspnoea, clear chest on auscultation or very minimal auscultatory signs in proportion to the degree of dyspnoea, and a chest radiograph showing diffuse interstitial infiltrate. None of the patients studied were on highly active antiretroviral therapy (HAART) nor were any of them on trimethoprim-sulphamethaxazole prophylaxis for PCP. Signed informed consent for fibre-optic bronchoscopy was obtained in 50 patients. Oropharyngeal samples and BAL from 16 HIV seronegative subjects with respiratory symptoms and an abnormal chest radiograph were used as negative controls.

Oropharyngeal samples were obtained in all 50 patients. Forty eight patients were able to tolerate fibreoptic bronchoscopy and a transbronchial biopsy was obtained in 35 and BAL in 48 patients. It was not possible to obtain lung biopsy samples as well as BAL fluid in all

the patients. Two patients had desaturation or intolerance of the bronchoscope. In 13 patients a transbronchial biopsy was contraindicated.

1 ml of sample was used to extract DNA. The primers used in the PCR reaction were based on the gene encoding the large subunit mitochondrial ribosomal RNA (mt LSU rRNA). The details of DNA extraction, primers, PCR and histological examination appear in Chapter 2.

A nested PCR was used to amplify a 205 base pair product and visualization was performed on a 2 % agarose gel stained with ethidium bromide. Negative and positive controls were included in each run. The test was considered positive for *Pneumocystis jirovecii* if a 205 base pair product was visible after the nested step.

5.4 RESULTS

Positive histology for PCP was obtained in 16 of the 35 subjects (46 %). The remaining 19 PCP negative reports were as follows: normal lung (4), lymphocytic interstitial pneumonitis (1), non-specific mixed inflammation (11), undifferentiated carcinoma (1), chronic inflammation with bilharzia (1) and fibro-fatty tissue (1). Oropharyngeal washings yielded positive results for *Pneumocystis jirovecii* in 7 of the 16 patients who had histologically proven PCP giving a sensitivity of 44 %. Four patients who had negative histology for PCP had positive PCR results in oropharyngeal samples. Compared with histology, the PCR test for *Pneumocystis jirovecii* DNA in oropharyngeal washes had a

specificity of 79 %, a positive predictive value of 64 % and a negative predictive value of 63 % (Table 5.1)

Forty eight patients had bronchoscopy and BAL performed. Thirty-five (73 %) had a positive result for PCP on lavage fluid analysis by PCR. The relative yield of oropharyngeal washings compared to bronchoalveolar lavage fluid was 40 %. Compared with BAL fluid, oropharyngeal washes had a sensitivity of 40 %, specificity of 77 %, positive predictive value of 82 % and a negative predictive value of 32 % (Table 5.2)

Fifteen HIV seronegative control subjects had fiberoptic bronchoscopy and BAL and were negative for *Pneumocystis jirovecii* DNA by PCR. One subject had sarcoidosis, one had squamous cell carcinoma, 9 had non-specific inflammation and/or interstitial fibrosis and in the remaining 4, transbronchial biopsies could not be performed. None were positive for *Pneumocystis jirovecii* in oropharyngeal washes. Comparison between the use of induced sputum and BAL as diagnostic samples for nested PCR showed concordance between the two samples to be 100 % in 7 patients (Table 5.3)

Table 5.1: 2x2 table of oropharyngeal washes versus histology in thirty-five subjects

Oropharyngeal Washes	Histology		
	Positive	Negative	Total
Positive	7	4	11
Negative	9	15	24
Total	16	19	35

Sensitivity=44 %; specificity=79 %

Table 5.2: 2x2 table comparing BAL with or pharyngeal washes in all subjects

Oropharyngeal Washes	BAL		
	Positive	Negative	Total
Positive	14	3	17
Negative	21	10	31
Total	35	13	48

Sensitivity=40 %; specificity=77

Table 5.3: 2x2 table comparing BAL and induced sputum samples for nested PCR.

Induced sputum	BAL		
	Positive	Negative	Total
Positive	5	0	5
Negative	0	2	2
Total	5	2	7

Concordance=100 %

5.5 DISCUSSION

The most accurate methods for diagnosing PCP are BAL analysis or lung biopsy. Both are invasive and possible only in stable patients. The sensitivities of the two methods have been reported to be between 98 % and 100 % (Pitchenik et al, 1986; Leigh et al, 1989; Metersky et al 1998). Induced sputum, a non invasive method of diagnosing PCP, has a sensitivity of approximately 50 % but unfortunately is contraindicated in seriously ill patients. Expecterated sputum is inadequate for the diagnosis of PCP with a sensitivity of only 34 % (Leigh et al, 1989; Wakefield et al, 1990). Empiric treatment is not always desirable, and some patients have atypical presentations.

This study has shown that that the application of this set of primers had a low sensitivity and specificity when compared to lung biopsy and BAL for the diagnosis of PCP. The use

of PCR has resulted in increased detection of *Pneumocystis carinii* from BAL fluid, induced sputum and expectorated sputum (Tuncer et al, 1998). The utility of DNA amplification to detect *Pneumocystis jirovecii* in oropharyngeal washes has previously been described by Wakefield and co-workers who reported a sensitivity of 56 % (Wakefield et al, 1990; Wakefield et al, 1993; Miller, 1997) in comparison to the standard investigation of microscopy after methenamine silver staining of the bronchoscopic lavage sample. To date no study has compared the sensitivity and specificity of oropharyngeal washes to histology.

This study has shown the sensitivity of DNA amplification in oropharyngeal washes to be 44 % using histology as the gold standard and 40 % using BAL.

Positive oropharyngeal washings were obtained in 4 subjects; all 4 had negative histology for PCP. These were possibly false positives. Alternatively these were true positives; the histology could have been negative due to a sampling error as blind biopsies were obtained. A rerun of the PCR in the 4 samples together with a re-examination of the respective histology samples was not done. This might have clarified the situation better.

Whilst an oropharyngeal PCR positive result is of value, a diagnosis of PCP cannot be excluded if the test is negative. Although PCP is predominantly an alveolar disease, the detection of organisms in oropharyngeal samples could be explained on the basis of some organisms reaching the upper airways during coughing or invasion of the upper airways with severe disease. In a study of 150 HIV infected African children with pneumonia from Malawi (Graham et al, 2000), *Pneumocystis jirovecii* was isolated from nasopharyngeal aspirates in 16 cases; thus the organisms are capable of colonizing the upper airways. The

probability of isolating organisms from the upper airways and mouth may depend on the depth and frequency of the cough, the severity of the disease as well as the degree of immunocompromise.

Sampling the oropharynx in the HIV seropositive patient is appealing. The technique is simple, has no risk to the patient, and is quick. PCR is now widely available and readily accomplished. Wakefield and colleagues have shown improved sensitivity of up to 78 % with oligoblotting (Wakefield et al, 1993). Refinements of the methods of sample collection, storage and laboratory techniques can be expected to result in higher sensitivities. Concern has been raised regarding problems with false positive results for control patients (Khan et al, 1999). This may limit the applicability of this method.

The notion that PCP is rare in African patients afflicted with HIV/AIDS is no longer true. Our study has shown that 46 % to 73 % of HIV infected subjects presenting with respiratory symptoms and diffuse pulmonary interstitial infiltrates may have PCP. This is important when drawing up treatment protocols for the region in patients presenting with such clinical and radiological features. This cannot be extrapolated to other patients with CAP (where the prevalence should be expected to be lower) because of the selection bias in this cohort.

In conclusion, the use of nested PCR, under the above conditions, to detect *Pneumocystis jirovecii* in oropharyngeal washings from HIV infected subjects has a low sensitivity and specificity for the diagnosis of PCP. Future studies should focus on improving its sensitivity and specificity.

CHAPTER 6

A prospective analysis of initial antibiotic therapy in severe

CAP: Evaluation of adherence to the SATS guidelines.

6.1 Summary

Aims of study

1. To evaluate physician compliance with the South African CAP practice guidelines.
2. To evaluate the usefulness of the guidelines in reducing length of stay and mortality in hospitalized patients with severe CAP.

Methods

Four hundred and thirty patients who presented to acute medical admissions between June 2000 and October 2001 with CAP were recruited into the study. The following data were collected on admission: age, a history of chronic liver and renal disease, cardiac failure, diabetes mellitus; confusion, respiratory rate, hypotension, oxygen saturation and partial pressure of arterial oxygen (90 % or < 60 mmHg), temperature greater than 38,3⁰ C and radiological involvement of two or more lobes. The presence of two or more of these parameters was considered as denoting severe CAP at presentation and before biochemical parameters were available. The admitting physician and team decided on the initial empiric antibiotic(s) administered without any influence from the investigators. Data on antibiotics administered, outcome and length of stay was retrieved from patients' records.

The first day of stay was taken as day 1. The September 1996 South African Thoracic Society (SATS) guidelines (Bateman et al, 1996) on the management of CAP were used to evaluate physician compliance. Patients with suspected or proven PCP, proven pulmonary tuberculosis, lung cancer, fungal and viral pneumonia were excluded from the final analysis. This retrospective exclusion was necessary as the SATS guidelines as defined are for bacterial CAP. The treatment of pulmonary tuberculosis, viral and fungal pneumonia as

well as that of PCP was not covered by the guidelines. The treatment of these pneumonias is completely different from the treatment of bacterial or presumed bacterial CAP.

Results

Two hundred and thirty seven out of four hundred and thirty cases were eligible for analysis. One hundred and eighty two patients (84 %) had two or more markers of severe pneumonia. Fourteen of the 182 patients (8 %) had initial antibiotic therapy administered according to South African guidelines and the remaining 92 % (168) did not. Total mortality was 20 % (36 patients). There was no statistical difference in length of stay between the two groups (14 vs 12 days; $p = 1.0000$; odds ratio 1.167; 95 % CI 0.3926 - 3.467) or in mortality (28.5 % vs 19 %; $p = 0.3549$; odds ratio 1.667; 95 % CI 0.667 - 4.161)

Conclusion

The majority of patients with severe CAP at King Edward VIII hospital are not treated with antibiotic regimens recommended in the South African guidelines. Compliance with antibiotic regimens for severe CAP did not result in a shortening of the duration of hospital stay or a reduction in mortality.

6.2 Introduction

The choice of antibiotics in the initial treatment of CAP should cover the most likely pathogens and the pathogens most likely to cause severe disease. Guidelines have been

6.3 Patients and methods

The study was performed between June 2000 and October 2001. Details of patient recruitment appear in Chapter 2. The admitting physician determined the initial antibiotic(s) administered. Severe CAP was defined as the presence of two or more of the following : age greater than 60 years, co-morbid disease (neoplastic disease, chronic renal failure, chronic liver disease, diabetes mellitus, heart failure), confusion, cyanosis, systolic blood pressure < 90 mmHg or diastolic blood pressure < 60 mmHg, respiratory rate \geq 30 breaths per minute, temperature $> 38.3^{\circ}$ C, involvement of two or more lobes on the chest radiograph, arterial partial pressure of oxygen < 60 mmHg, white blood cell count $< 4 \times 10^9$ cells/mm³ or $> 30 \times 10^9$ cells/mm³, urea > 7 mmol/l and albumin < 30 mmol/l. Patients who had clinical and radiological evidence of PCP and who were started on high dose trimethoprim-sulphamethoxazole, or pulmonary tuberculosis were excluded. Subjects who did not meet the severity criteria as defined were also excluded.

Statistical Analysis

The length of stay in days and the in hospital mortality rates were compared between patients whose antimicrobial therapy for severe CAP was consistent with South African guidelines and those in which it was inconsistent. The unpaired t test and the Fisher's Exact Test were used in analyzing the data.

6.4 Results

Four hundred and thirty in- patients with CAP were recruited. One hundred and eighty two fulfilled the study criteria. One hundred and fifty-three of the 182 patients (84 %) were HIV infected. The excluded 248 patients did not meet the criteria for severe disease, or

6.3 Patients and methods

The study was performed between June 2000 and October 2001. Details of patient recruitment appear in Chapter 2. The admitting physician determined the initial antibiotic(s) administered. Severe CAP was defined as the presence of two or more of the following : age greater than 60 years, co-morbid disease (neoplastic disease, chronic renal failure, chronic liver disease, diabetes mellitus, heart failure), confusion, cyanosis, systolic blood pressure < 90 mmHg or diastolic blood pressure < 60 mmHg, respiratory rate ≥ 30 breaths per minute, temperature $> 38.3^{\circ}\text{C}$, involvement of two or more lobes on the chest radiograph, arterial partial pressure of oxygen < 60 mmHg, white blood cell count $< 4 \times 10^9$ cells/mm³ or $> 30 \times 10^9$ cells/mm³, urea > 7 mmol/l and albumin < 30 mmol/l. Patients who had clinical and radiological evidence of PCP and who were started on high dose trimethoprim-sulphamethoxazole, or pulmonary tuberculosis were excluded. Subjects who did not meet the severity criteria as defined were also excluded.

Statistical Analysis

The length of stay in days and the in hospital mortality rates were compared between patients whose antimicrobial therapy for severe CAP was consistent with South African guidelines and those in which it was inconsistent. The unpaired t test and the Fisher's Exact Test were used in analyzing the data.

6.4 Results

Four hundred and thirty in- patients with CAP were recruited. One hundred and eighty two fulfilled the study criteria. One hundred and fifty-three of the 182 patients (84 %) were HIV infected. The excluded 248 patients did not meet the criteria for severe disease, or

had proven *Mycobacterium tuberculosis*, PCP, fungal pneumonia or were treated empirically for PCP or pulmonary tuberculosis. The proportion of patients with each parameter considered as a marker of severe disease were: age > 60 years: 2 % of patients, comorbid disease: 9 %, confusion: 0 %, hypotension: 1 %, involvement of 2 or more lobes: 58 %, respiratory rate ≥ 30 breaths per minute: 59 %, temperature $\geq 38.3^{\circ}\text{C}$: 53 %, partial pressure of arterial oxygen < 60 mmHg: 8 %, white cell count < 4×10^9 cells/mm³ or > 30×10^9 cells/mm³: 9 %, urea > 7 mmol/l: 49 % and albumin < 30 mmol/l: 82 % (Table 6.1)

Table 6.1 Proportion of patients with each parameter of severe disease (Each patient had two or more markers)

Parameter	Number (N)	% of Patients
Age > 60 years	4	2
Co-morbid disease	16	9
Confusion	0	0
Hypotension	2	1
Radiographic involvement of 2 or more lobes	106	58
Respiratory rate ≥ 30 breaths /minute	107	59
Temperature $> 38.3^{\circ}\text{C}$	96	53
Pleural effusion	0	0
PaO ₂ < 60 mmHg	15	8
WBC < 4 or > 30×10^9 cells	17	9
Urea > 7 mmol/l	90	49
Albumin < 30 mmol/l	150	82

Empiric antibiotic treatment for severe CAP consistent with South African guidelines was administered to 14 patients (8 %) only. The remaining 168 patients (92 %) were given treatment that was inconsistent with the guidelines. In the cohort of patients in whom the guidelines were followed, 9 subjects received intravenous second generation cephalosporin and an aminoglycoside, 4 patients had intravenous amoxicillin-clavulanic acid and an

aminoglycoside, and one patient intravenous second generation cephalosporin and a macrolide. In the cohort in which the guidelines were not followed, antibiotic regimens administered were: intravenous second generation cephalosporin alone in 85 patients, intravenous penicillin G (28 patients), intravenous amoxicillin–clavulanic acid (28 patients), intravenous penicillin G and an aminoglycoside (8 patients), intravenous penicillin G and a fluoroquinolone (1 patient), intravenous penicillin G and chloramphenicol (1 patient), intravenous piperacillin and an aminoglycoside (1 patient), intravenous third generation cephalosporin (7 patients), intravenous flucloxacillin and third generation cephalosporin (1 patient), intravenous amoxicillin-clavulanic acid and a fluoroquinolone (1 patient), piperacillin and a fluoroquinolone (1 patient), a second generation cephalosporin and fluoroquinolone (1 patient) and oral amoxycillin in 3 patients (Table 6.2).

Table 6.2 Physicians’ antibiotic choices in the cohort of patients in which the South African guidelines were not followed

Antibiotic choice	Number of patients	% Of total
I.V 2 nd generation cephalosporin	85	50.5
I.V penicillin G	28	17
I.V amoxicillin-clavulanic acid	28	17
I.V penicillin G and aminoglycoside	8	5
I.V 3 rd generation cephalosporin	7	4
Oral amoxycillin	3	2
IV penicillin G and fluoroquinolone	1	0.5
IV penicillin G and chloramphenicol	1	0.5
IV piperacillin and aminoglycoside	1	0.5
IV piperacillin and fluoroquinolone	1	0.5
IV flucloxacillin and 3 rd generation cephalosporin	1	0.5
Oral amoxycillin and metronidazole	1	0.5
IV 2 nd generation cephalosporin and fluoroquine	1	0.5
IV amoxicillin –clavulanic acid and fluoroquinolone	1	0.5
Oral erythromycin	1	0.5

Thirty-six patients died, resulting in an overall mortality rate of 20 %. Four patients (28.5 %) died in the group that received treatment consistent with the South African guidelines while 32 patients (19 %) died in the cohort whose antibiotic regimens were not consistent with the guidelines. The difference in mortality was not statistically significant ($p = 0.7106$).

A total of 113 bacterial isolates were identified, 50 % of these were *Streptococcus pneumoniae*. Mortality was associated with *Streptococcus pneumoniae* in 9 patients, *mycoplasma pneumoniae* 4 patients, *Pseudomonas aeruginosa* in 2 patients, *Chlamydia pneumoniae* in 2 patients and *Klebsiella pneumoniae* with 1 patient. No organisms were identified in the remaining 18 deaths (Table 6.3)

Table 6.3 Bacterial organisms isolated in 182 patients with CAP

Organism	Number of isolates	% Of total
<i>Streptococcus pneumoniae</i>	56	50
<i>Mycoplasma pneumoniae</i>	15	13
<i>Staphylococcus aureus</i>	12	11
<i>Chlamydia pneumoniae</i>	9	8
<i>Haemophillus influenzae</i>	7	6
<i>Pseudomonas aeruginosa</i>	4	3.5
<i>Klebsiella pneumoniae</i>	3	2.5
Bacillus species	3	2.5
<i>Eschericia coli</i>	2	1.5
<i>Legionella pneumophilla</i>	1	1
Gram negative bacilli	1	1

There was no statistically significant difference in the mean length of hospital stay between the group in which the initial empiric antibiotic therapy was consistent with South African

guidelines and the group in which the guidelines were not adhered to (14 days vs 11.7 days; $p = 0.3995$) (Table 6.4).

Table 6.4 Length of stay in days in 146 patients who survived

Parameter	South African guidelines followed	South African guidelines not followed
Mean length of stay in days	14	11.732
Std deviation	5.944	8.323
Std error of the mean	1.880	0.7085
Minimum	7	3
Maximum	29	68
Median	13	10
Lower 95 % CI	9.748	10.343
Upper 95 % CI	18.252	13.121

6.5 Discussion

Clinical guidelines for the management of CAP in South Africa were developed by Working Groups of the South African Pulmonology Society and the Antibiotic Study Group of South Africa (Bateman et al, 1996). In developing these recommendations reference was made to Canadian, British and American guidelines. The Medical Association of South Africa (now the South African Medical Association) endorsed the recommendations. This study provides the first objective analysis of the impact these guidelines have had on physician practice, length of stay and mortality at a tertiary hospital.

The day of admission was taken as day 1 of the duration of hospital stay. That was the point at which empiric antibiotic treatment was commenced. Therefore the assessment of response to treatment as judged by the length of stay in hospital was measured from day

one. This is justified despite the variable time of presentation by the patients. In order for the study to reflect data on patients with bacterial pneumonia only (proven or presumed), patients with tuberculosis, PCP and fungi were excluded.

The results indicate that most patients with severe CAP were treated with antibiotic regimens that were inconsistent with South African guidelines (92 %). The majority of cases were treated with a second generation cephalosporin alone (50.5 %), intravenous penicillin alone (17 %), or intravenous amoxycillin-clavulanic acid. There was virtually no cover for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* except in one patient who received a second-generation cephalosporin and a macrolide. The practice of not covering for the so called “atypical” pathogens probably stems from lack of clear evidence as to whether they are common pathogens or not in patients with CAP in South Africa (Prout et al, 1983; Potgieter et al, 1992; Maartens et al, 1994). In this study *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* accounted for 13 %, 8 % and 1 % of isolates respectively. Taken together, the data from our study suggest that up to 20 % of patients with severe CAP may have an atypical pathogen. These data are important in further elucidating the role of atypical pathogens in the epidemiology of CAP in South Africa. *Streptococcus pneumoniae* was the commonest cause of severe CAP, and this is consistent with most published data.

There was no statistically significant difference in the length of stay between patients whose treatment was consistent with South African guidelines and the group whose treatment was inconsistent with the recommendations. However, as only 14 out of 182 patients were treated according to guidelines, the sample size may be small to detect meaningful difference. The length of stay in both groups (14 days and 12 days

respectively) is a reflection of the severity of illness combined with socio-economic deprivation. Studies of length of stay in hospitalized patients with CAP are scarce. Two studies from the USA reported lengths of stay of 8.5 and 6.3 days (Rosenthal et al, 1997; Health Care Management Guidelines, 1995). Both studies looked at hospitalized patients with CAP but the severity of disease varied. Besides the severity of illness, the high HIV sero-positivity rate of 84 % is an important consideration. Patients tend to have a multiplicity of problems such as wasting, general debilitation and dehydration.

Mortality was 28.5 % and 19 % in the patients who were given treatment consistent with and inconsistent with South African guidelines respectively. Although the group in which the guidelines were not followed appeared to have had a better outcome, this difference did not reach statistical significance ($p = 0.7106$). One factor that might have influenced these results is the small number of patients in the group that received treatment according to the published guidelines. A potential weakness of this study was the non application of multiple logistic regression models to the confounding variable to adjust for the differences in mortality and length of stay in the two groups.

Published mortality figures in hospitalized immunocompetent patients with CAP have ranged from 2 % to 30 % with an average rate of 14 % (Bartlett et al, 1998). Koulla Shiro et al, 1996, reported mortality rates of 7.1 % and 7.3 % in hospitalized HIV infected and non-HIV infected patients respectively. A Spanish study of HIV infected patients with reported mortality rates of 3.5 % and 13.1 % in those with moderate and severe CAP respectively (Cordero et al, 2000).

The study has shown that the South African guidelines have failed to significantly influence medical practice at a tertiary teaching hospital. There are several explanations (Lomas et al, 1989; Greco et al, 1993; Delamothe, 1993; Fein et al, 1995). These include lack of awareness, attitude, knowledge of the guidelines and local barriers to implementation of the guidelines. In addition, physicians may feel that guidelines restrict their autonomy, freedom and clinical judgement. Possible solutions for barriers to change include continuing medical education, feedback and physicians' participation in efforts to have guidelines adopted and implemented, participation of administrators as well as regular guideline revision and auditing. Guidelines need to be flexible and to be scientifically validated.

This study brings into focus the the issue of instutional policy regarding the use of antimicrobials. There is no institutional policy on the use of antibiotics in CAP within the medical domain at King Edward VII hospital. The failure to provide clear policy may be due to various reasons including ignorance of the need and advantages of well formulated policies, fear of alienating professionals, a lack of or shortage of good managers, inadequate financial resources which might make such policies unworkable, and bureaucratic and political interference. Sound working relationships between institutional managers and clinicians are necessary for any policy to work. Erratic supplies of drugs would render any institutional policies unworkable. In the light of the data on compliance from this study, its important to get some feedback from the clinicians regarding guidelines in general and the SATS CAP guidelines in particular. This could be a follow up study in the form of a questionnaire. Valuable data about knowledge, attitudes, fears, concerns and antibiotic practising habits could emanate from such a study. The study could could provide answers for the poor adherence to the guidelines.

The SATS CAP guidelines were recently revised. Clear, workable strategies need to be devised to disseminate these guidelines to practising clinicians in an efficient and cost effective manner. If the data from this study is a reflection of practice country-wide, a lot of work would be required to change physicians' practising habits. Although it may be argued that mortality is not necessarily altered by following guidelines, there are equally compelling reasons for the issue of guidelines to be pursued vigorously. These are cost effectiveness, rationale prescribing and minimizing the development of antibiotic resistance, among other reasons. Lastly, the revised SATS CAP guidelines should put emphasis on the emergence of tuberculosis and PCP as common pathogens in the light of the HIV epidemic. Laboratory, technical and human resources are not available at many hospitals country-wide. Invasive and semi-invasive procedures required to diagnose PCP cannot be performed at most hospitals. Patients with clinical features of immunocompromise or a history of HIV infection presenting with bilateral interstitial or alveolar infiltrate should be covered for PCP. In patients with CAP not responding to appropriate antibiotic cover, and samples of blood and sputum have been submitted for bacteria nad mycobacteria, there should be a low threshold to treat for tuberculosis. Careful observation for clinical response is mandatory.

In conclusion, this study has demonstrated the importance of validating the clinical usefulness of CAP guidelines. The majority of patients with severe CAP are prescribed antibiotic treatment that is not consistent with South African guidelines at King Edward VIII hospital. This may reflect a general disregard for CAP antibiotic guidelines elsewhere in South Africa. Guidelines may have no significant effect on length of stay or mortality as demonstrated in this study. Future studies should address the reasons for the non-compliance and the economic implications.

CHAPTER 7

Validation of the pneumonia risk classification Fine Score in HIV seropositive subjects

7.1 Summary

Aim of Study

To validate the Fine Score severity criteria for CAP in HIV infected subjects in a resource limited setting.

Methods

Four hundred and thirty patients with CAP were studied. Demographic data, comorbid disease, clinical parameters relating to mental status, pulse rate, blood pressure, respiratory rate, temperature, radiographic and laboratory data were collected to calculate the Fine score.

Results

Two hundred and eighty nine patients were analysed after excluding *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, fungal and viral pneumonias. Two hundred and forty patients were HIV infected (83 %). The mortality rates per risk class were 13 % and 20.6 % for Grade II; 19 % and 50 % for Grade III; 38.9 % and 42 % for Grade IV ; 100 % and 100 % for Grade V in the HIV and non-HIV infected cohorts respectively. There was a significant positive correlation between the Fine Score and mortality in both the HIV positive and HIV negative cohorts ($p < 0.0001$). There was no significant difference in mortality between the two groups ($p = 0.8766$).

Conclusion

The Fine Score is useful in predicting mortality in HIV infected subjects with CAP.

7.2 Introduction

Numerous studies have addressed severity criteria of CAP in non-HIV infected patients (Woodhead et al, 1985; Tores et al, 1991; Rello et al, 1993; Moine et al, 1994; Leroy et al, 1995; Feldman et al, 1995; Santiago et al, 1998). Although there are no universally accepted criteria for severe CAP, a prediction rule derived and validated by Fine et al (1997), is widely applied in addressing physicians' dilemmas about outpatient treatment, admission and aggressive management including intensive care.

CAP is a major complication of infection with HIV. The incidence of CAP in HIV seropositive subjects ranges between 1.93 and 19.2 per 100 patient years with a mortality of 2.6 % to 27 % (Selwyn et al, 1988; Magnenat et al, 1991; Wallace et al, 1993; Hirschitlik et al, 1995; Wallace et al, 1997).

Studies of severity assessment and mortality factors in HIV associated CAP are scarce. In the only major study addressing this issue (Cordero et al, 2000), three factors were independently found to be associated with mortality: CD4 cell count below 100/microlitre, pleural effusion, cavities, multi-lobar infiltrates (radiologic progression of disease) and shock. The ATS criteria for severe CAP were validated in that study, with patients who fulfilled the criteria having a longer duration of hospital stay and a higher attributable mortality of 13.1 % compared to those who did not. The Fine Score has not been evaluated in CAP associated with HIV infection.

7.3 Patients and methods

Patient selection

All patients presenting with CAP between June 2000 and October 2001 were recruited. Demographic and clinical data were collected at the time of admission. Chest radiographs were obtained in all patients. Laboratory and microbiological investigations were performed as described in chapter 2. Details regarding calculation of the Fine Score appear in appendix 2.

Retrospectively, patients with a diagnosis of *Mycobacterium tuberculosis*, PCP, fungal, viral pneumonia, carcinoma of the bronchus and unknown HIV sero-status were excluded. The Fine Score was applied to categorize the patients with CAP into Grade II – IV.

7.4 Results

Two hundred and eighty nine out of four hundred and thirty patients met the inclusion criteria for the final data analysis. Two hundred and forty subjects were HIV seropositive (83 %). Organisms were isolated in 139 patients (48 %). *Streptococcus pneumoniae* was the commonest cause of CAP in both HIV and non-HIV infected patients, accounting for 50 % and 33 % of isolates respectively and 46 % overall (Table 7.1).

The combined mortality was 20 %. In the HIV infected cohort, 42 patients died (17.5 % mortality), compared with 16 deaths (32.6 % mortality) in the non- HIV infected group. The proportions of patients in the various grades are shown in table 7.2.

Table 7.1: Distribution of organisms in 139 patients in whom organisms were isolated

Organism	HIV positive n (%)	HIV negative n (%)	Total n (%)
<i>Streptococcus pneumoniae</i>	60 (50)	10 (23)	70 (46)
<i>Mycoplasma pneumoniae</i>	24 (20)	5 (17)	29 (19)
<i>Staphylococcus aureus</i>	12 (10)	4 (13)	16 (11)
<i>Chlamydia pneumoniae</i>	9 (7)	1 (3)	10 (7)
<i>Haemophilus influenzae</i>	6 (5)	4 (13)	10 (7)
<i>Escherichia coli</i>	3 (2)	1 (3)	4 (2.5)
<i>Klebsiella pneumoniae</i>	2 (1)	1 (3)	3 (2)
<i>Legionella pneumophilla</i>	1 (1)	0 (0)	1 (0.5)
<i>Moraxella catarrhalis</i>	1 (1)	0 (0)	1 (0.5)
<i>Pseudomonas aeruginosa</i>	1 (1)	2 (7.5)	3 (2)
Bacillus species	1 (1)	2 (7.5)	3 (2)
Gram neg bacilli	1 (1)	0 (0)	3 (0.5)
TOTAL	121	30	151

n refers to the number of patients.

Table 7.2 Proportion of patients in each Fine Score grade

Fine Score	HIV infected N (%)	Non-HIV infected N (%)	Total N (%)
Grade II	172 (72)	29 (59)	201 (70)
Grade III	47 (20)	12 (25)	59 (20)
Grade IV	18 (7)	7 (14)	25 (9)
Grade V	3 (1)	1 (2)	4 (1)
Total	240 (100)	49 (100)	289 (100)

The overall mortality was 58 deaths (20 %) with mortality rates of 14 %, 25.4 %, 40 %, 100 % in Grade II, Grade III, Grade IV and Grade V respectively (Table 7.3)

Table 7.3 Overall mortality in each grade

Fine Score Grade	No. of deaths/No. in grade	Mortality %
Grade II	29/201	14%
Grade III	15/59	25.4%
Grade IV	10/25	40%
Grade V	4/4	100%

Table 7.4: Comparison of mortality in HIV infected and non-HIV infected patients according to the Fine Score grade

Pneumonia Risk Classification Fine Score Grade	HIV Seropositive				HIV Seronegative			
	II	III	IV	V	II	III	IV	V
Number of patients	172	47	18	3	29	12	7	1
Number of deaths	23	9	7	3	6	6	3	1
Mortality (%)	13.4	19.1	38.9	100	20.6	50.0	42.8	100
p – Value	p < 0.0001				p < 0.0001			

The p value refers to comparison of mortality rates between the different grades in each cohort. This is the chi square p value for overall association between pneumonia risk category and mortality. It can be seen from the table that the proportion of mortality increases as the category increases. This is confirmed with a chi square test for trend.

There was strong correlation between mortality and the Fine Score grade for each cohort (p < 0.0001). (Table 9.4). When mortality rates per risk class were compared, no significant differences emerged between the two cohorts (p = 0.8062). In addition, there was no significant difference in overall mortality between the two groups (p = 0.8766).

Deaths in the HIV infected group were due to *Streptococcus pneumoniae* in 9 subjects, *Staphylococcus aureus* in 9, *Mycoplasma pneumoniae* in 5, coinfection with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in one case and coinfection with *Klebsiella pneumoniae* and *Streptococcus pneumoniae* in another. No pathogens were isolated in the remaining 17 deaths. Eight of the 16 deaths in the non-HIV infected group were associated with *Streptococcus pneumoniae* (2), *Staphylococcus aureus* (3), coinfection with

Streptococcus pneumoniae and *Staphylococcus aureus* (1), *Pseudomonas aeruginosa* (1), and coinfection with *Pseudomonas aeruginosa* and *Chlamydia pneumoniae* (1).

7.5 Discussion

This is the first study to validate the Fine Score in HIV infected subjects with CAP in a resource poor setting. There is strong correlation between mortality and the Fine Score in both HIV and non-HIV infected patients with CAP. The mortality rates for patients in Grade II and Grade III were 13.4 % and 19.11 %, and 20.6 % and 50 % in HIV positive and HIV negative patients respectively. This is a considerably higher mortality than results reported by Fine et al (1997) in which the mortality rates ranged from 0.6 % to 0.7 % (class II) and from 0.9 % to 2.8 % for class III. In that study, patients in class IV and V had mortality which ranged from 8.2 % to 9 % and 27.1 % to 31.1 % respectively. In contrast, mortality for class IV and V in our study was even higher at 38.9 % and 100 % (HIV positive) and 42.8 % and 100 % in the nonHIV infected cohort.

The mortality rates in this study may reflect, in general, the poor socio-economic status of our patient population. Although the socio-economic status of the patients enrolled could not be quantified in monetary terms, all were Black Africans from the townships and informal settlements or rural areas around Durban. It is a socio-economic fact that the poorest South Africans are from this racial group and from these settlements, formal and informal. The Fine scoring system does not take into account cachexia. It is a risk factor for mortality in African patients with pneumonia (Grant et al, 1998). It should be modified and cachexia incorporated.

These high mortality data are an indication of the need for aggressive management of CAP including high quality nursing care, optimal fluid administration and nutrition as well as oxygen administration in sick patients. This must be additional to appropriate antibiotics administered in the recommended combinations, dosages and routes. Constant vigilance is required for high risk patients and the role of physiotherapy needs to be explored. The perennial staff shortages, compounded by the huge burden of increasing patient numbers in the public sector, must be addressed if mortality figures are going to drop significantly to the low levels seen in North America and Western Europe.

Analysis of mortality per risk class as well as overall mortality showed no significant differences between the two groups. In most studies mortality among hospitalized patients without HIV infection has ranged between 2 % and 30 % (Bartlett et al, 1998). Reported in-hospital mortality in patients with HIV infection has ranged between 2.6 % and 27 % (Teira et al, 1992; Falco et al, 1994; Hirschitnik et al, 1995; Boschini et al, 1996; Tumbarello et al, 1998). The high mortality seen in the HIV seronegative cohort may be a chance finding, or may be due to the small numbers as there were no obvious confounding variables in the group. There is a tendency to treat HIV infected patients with antibiotics that possess a much broader spectrum of cover as well as the liberal use of high dose trimethoprim-sulphamethoxazole. This may have positive survival benefits.

Patients with tuberculosis, PCP, viral and fungal pneumonia were excluded from the final analysis as the Fine score was derived from patients with bacterial CAP. *Streptococcus pneumoniae* was not only the commonest pathogen isolated, but also the organism associated with the highest mortality. Of even greater concern was the mortality associated with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* particularly since the role

played by the so-called atypical bacteria in the aetiology of CAP in South Africa is controversial (Myburg et al, 1993; Maartens et al, 1994; Potgieter, 1995; Maartens, 1995).

There is need for both aggressive and novel forms of therapy in CAP as demonstrated by the relatively high mortality in risk class II and III, classes which according to the original recommendations by Fine and colleagues could safely be managed as out patients. The practice of not routinely prescribing antibiotic cover for atypical bacteria in our region may be a contributing factor to morbidity and mortality. This policy may need revision.

It is essential to have simple cost effective methods of identifying patients that are likely to have a poor outcome particularly in HIV infected subjects who have traditionally been excluded from studies of severity and mortality. The results of this study have shown that the Fine Score has merit for use in patients infected with HIV. It is simple to apply, and cost-effective as it does not rely on expensive tests like the CD4 count. The score can be applied at first levels of care like district hospitals in resource poor countries allowing for an appropriate management strategy to be implemented. Correlation between the CD4 count and the Fine score should be researched in the future. This could not be studied due to financial constraints. Such a study would enable the impact of the severity of immunosuppression on outcome to be assessed.

In conclusion, the Fine Score is a useful tool for predicting mortality in HIV infected patients with CAP. It can be easily applied in a resource poor setting.

CHAPTER 8

**A prospective study of the relationship between markers of
disease severity and yield from blood cultures in HIV and
non-HIV infected subjects with CAP**

8.1 Summary

Aims of study

1. To determine the relationship between markers of disease severity and the yield from blood cultures.
2. To evaluate the impact of positive blood cultures on antibiotic prescribing.

Methods

Patients presenting with symptoms and signs compatible with CAP and requiring hospitalization were prospectively studied. At presentation, clinical parameters necessary for the calculation of the Fine Score were recorded. Aerobic and anaerobic blood cultures were performed before the administration of antibiotics. Details of methods are given in Chapter 2.

Results

One hundred and eighty one patients comprising 104 females and 77 males with a mean age of 33 years (range 18 – 72 years) were studied. Blood cultures were positive in 43 patients (24 %) of which 37 (20.4 %) were clinically significant isolates. *Streptococcus pneumoniae* was the commonest organism cultured (56 %) followed by *Staphylococcus aureus*. Correlation of the yield of blood cultures with the Fine Score, the respiratory rate, temperature and pulse rate did not show a statistically significant relationship ($p = 0.4860$, $p = 0.2270$, $p = 0.9317$ and $p = 0.8200$ respectively) although there was a tendency to higher yields the more severe the pneumonia. Nine patients (24 %) with positive cultures required a change in antibiotic therapy.

Conclusion

Blood cultures should be performed in hospitalized patients with CAP. The yields may be high across all grades of pneumonia severity. One in four patients with positive cultures may require a change in the antibiotic(s) administered.

8.2 Introduction

The value of performing blood cultures in hospitalized patients with CAP has been questioned (Chalasani et al, 1995; Ewig et al, 1996; Skerett, 1999; Bryan, 1999; Waterer et al, 2001; Wunderink et al, 2001). Despite the controversies, the ATS, the BTS, the CTS and the SATS all recommend routine blood cultures in hospitalized patients with CAP (British Thoracic Society, 1993; Mandell et al, 1993; Niederman et al, 1993; Feldman et al, 1996; Feldman, 1998). Few studies have looked at the yield, value and clinical utility of routine blood cultures in hospitalized patients with CAP.

The yield of blood cultures in hospitalized patients with CAP ranges between 4 % - 18 % (Skerett, 1999) with an average of 11 % (Bartlett et al, 1998). The yield in patients who have received antibiotics prior to hospitalization is less than 5 %. In patients without defined risk factors, blood cultures may not be cost-effective (Chalasani et al, 1995). Suggestions have been made to limit blood cultures to patients with Pneumonia Severity Index score grade IV and grade V as the value in mild to moderate cases may be minimal (Waterer et al, 2001). To date, this is the only study that has looked at the influence of the severity of CAP on the usefulness of blood cultures. The impact of blood cultures on antibiotic prescribing requires more studies.

HIV infection is a risk factor for bacterial pneumonia, with a rate of 5.5 per 100 person years compared to 0.9 per 100 person years in HIV sero-negative subjects (Hirschik et al, 1995). The risk of bacteraemic pneumonia in HIV disease is increased and may be as high as 26 % (Redd et al, 1990).

8.3 Patients and Methods

Between June 2000 and October 2001, 181 hospitalized subjects with CAP were recruited. All patients recruited into the study were eligible to have blood cultures performed unless blood culture specimen bottles were not available. The criteria for a diagnosis of CAP, blood cultures and calculation of the Fine score were performed as described in Chapter 2. A history of prior antibiotic use was noted at admission and blood cultures were performed before the administration of empiric antibiotics.

Data analysis

Differences in variables were compared using Chi-squared Test and Fischer's Exact Test.

A $p < 0.05$ was considered significant.

8.4 Results

Four hundred and thirty patients who fulfilled the study criteria were recruited into the study. One hundred and eighty one patients had blood cultures performed. In 249 patients, blood cultures could not be performed due to erratic shortages of blood culture bottles during the 16 month recruitment period. There were 77 males and 104 females with a mean age of 33 years (range 18 – 72 years). One hundred and thirty seven patients were HIV

infected (76 %) and 32 patients were HIV seronegative. The remaining 12 patients declined to give informed consent. None of the patients had received prior antibiotic therapy. Forty-three blood cultures were positive giving an overall positive rate of 24 %. However, 6 isolates were considered contaminants by the microbiologist (4 bacillus species, 1 corynebacterium species, 1 *Staphylococcus haemolyticus*), thus the clinically significant yield rate was 20.4 % (Table 8.1). The proportions of patients with positive blood cultures were 26 patients (19 %) and 7 patients (22 %) in the HIV seropositive and HIV seronegative groups respectively ($p = 1.000$).

Table 8.1 Organisms isolated in forty three positive blood cultures

Organism	Number of isolates	Clinically significant	Clinically not significant
<i>Streptococcus pneumoniae</i>	22	+	-
<i>Staphylococcus aureus</i>	8	+	-
Bacillus species	4	-	+
<i>Escherichia coli</i>	3	+	-
<i>Candida albicans</i>	1	+	-
Corynebacterium species	1	-	+
<i>Staphylococcus haemolyticus</i>	1	-	+
<i>Klebsiella pneumoniae</i>	1	+	-
Gram negative bacillus	1	+	-
<i>Cryptococcus neoformans</i>	1	+	-
Total	43	37	6

The differences in the proportion of patients with positive blood cultures per Fine score grade did not reach statistical significance ($p = 0.4860$) (Table 8.2). Analysis of the relationship of blood culture yield with temperature, pulse and respiratory rate did not show significant correlation (Tables 8.3; 8.4 and 8.5). While there was a positive correlation between the respiratory rate and the blood culture yield, this did not reach statistical significance ($p = 0.227$).

Table 8.2 Blood culture yield per Fine Score grade

Fine Score Grade	Number of patients	Number with significant positive cultures (%)
II	112	17 (15.2)
III	38	12 (31.6)
IV	14	3 (21.4)
V	1	1 (100)

Table 8.3 Temperature at presentation and the yield from blood cultures

Temperature on admission	Number of patients	Number of positive cultures (%)
< 38.3 °C	79	17 (21.5)
38.3 - 38.9 °C	25	4 (16)
> 39 °C	60	12 (20)

$p = 0.9317$. The p value is for the overall association between temperature and yield from blood cultures as statistically determined by chi square.

Table 8.4 Pulse at presentation and the yield from blood cultures

Pulse rate on admission	Number of patients	Number of positive blood cultures (%)
< 100 beats/min	40	8 (20)
100 – 120 beats/min	95	17 (17.9)
> 120 beats/min	28	8 (28.6)

p = 0.8200. This is the chi square p value for overall association between the pulse rate and the yield from blood cultures.

Table 8.5 Respiratory rate at presentation and the yield from blood cultures

Respiratory rate on admission	Number of patients	Number of positive blood cultures (%)
< 30 breaths/min	82	11 (13.4)
30 – 39 breaths/min	40	11 (27.5)
> 40 breaths/min	41	11 (26.8)

p = 0.2270. This is the chi square p value for overall association between the respiratory rate at admission and the yield from blood cultures.

There was no antibiotic change in all the patients in whom *Streptococcus pneumoniae* was isolated. The antibiotics were appropriate. The four patients with *Escherichia coli* (3) and *Klebsiella pneumoniae* (1) bacteraemia were already on potent gram negative cover and did not require a change in antibiotics. The patient whose blood culture grew candida albicans died within 24 hours of hospitalization. Bacterial cover had been given and it would have been necessary to change to an antifungal had the patient survived. The isolation of *Cryptococcus neoformans* in one patient necessitated a change of antibiotics from intravenous amoxi-clavulanic acid to fluconazole. Of the 8 cases of *Staphylococcal*

aureus bacteraemia, there was a change in the antibiotic regimen in 6. The antibiotic changes made are summarized in table 8.6

Table 8.6 Antibiotic changes necessitated by results of positive blood cultures

Initial empiric antibiotic regimen	Blood culture isolate	New antibiotic regimen
Intravenous penicillin G	<i>Staphylococcus aureus</i>	Intravenous cloxacillin
Intravenous amoxicillin-clavulanic acid	<i>Staphylococcus aureus</i>	Intravenous cloxacillin + fusidic acid
Trimethoprim-sulphamethoxazole	<i>Eschericia coli</i>	Intravenous cefuroxime
Intravenous cefuroxime	<i>Staphylococcus aureus</i>	Intravenous cloxacillin + fusidic acid
Intravenous ceftriaxone	<i>Eschericia coli</i>	Intravenous ciprofloxacin
Intravenous amoxicillin-clavulanic acid	<i>Cryptococcus neoformans</i>	Fluconazole
Intravenous penicillin G + oral metronidazole	<i>Staphylococcus aureus</i>	Intravenous amoxicillin-clavulanic acid
Intravenous amoxicillin-clavulanic acid	<i>Staphylococcus aureus</i>	Intravenous cloxacillin + fusidic acid
Intravenous penicillin G + gentamycin	<i>Staphylococcus aureus</i>	Intravenous cloxacillin + fusidic acid

Mortality was 21.5 % (39 patients, 10 with positive blood cultures). Twenty four of the 39 deaths were HIV infected subjects. The overall mortality in this group was 17.5 % and in the subgroup with bacteraemia 19 %. Of these, five patients had positive blood cultures (1 *Candida albicans*, 2 *Streptococcus pneumoniae*, 1 *Klebsiella pneumoniae* and 1 *Cryptococcus neoformans*). In the HIV sero-negative cohort, mortality was 37.5 % with patients who were bacteraemic having a mortality of 71 %.

8.5 Discussion

Blood culture yield in hospitalized patients with CAP averages 11 % (Marston et al, 1997; Bartlett et al, 1998) and ranges from 4 % - 18 % (Macfarlane et al, 1982; Lim et al, 1989;; Marie et al, 1989; 1990; Blanquer et al, 1991; ; Burman et al, 1991; Ishida et al 1991; Karalus et al, 1991; Bates et al, 1992; Bohte et al, 1995; Mundy et al; 1995; Fang et al, , 1998; Levy et al, 1998). The proportion of positive blood cultures in this study is higher compared to other series. Clinically significant positive blood cultures were obtained in 20.4 %. This may be partly explained by our patients presenting prior to the initiation of antibiotic therapy as well as to advances in laboratory culture techniques. Prior to 2000, the Bac T-alert blood culture system was used at our centre. The newer Bactec automated blood culture sytem has been in use from mid 2000.

No statistically significant differences in the proportion of blood culture yield per Fine score were found. This contrasts the results of a study by Waterer et al (1999) in which they concluded that blood cultures are of minimal value in mild to moderate CAP and that they should be limited to patients with Pneumonia Severity Indices grade IV or V unless there were other specific risk factors. Similar conclusions were made by Theethakari et al

(2001). In this study patients with pneumonia severity grade II had a 15.2 % positive blood culture yield. This is above published averages.

Mortality in hospitalized patients with CAP ranges between 2 % - 30 %. The mortality in our study was 21.5 %. The higher mortality rate of 37.5 % in the HIV seronegative group compared to 17.5 % in the HIV infected group may be due to the of the smaller sample size in the former. The liberal use of broad spectrum antibiotics, multiple antibiotics (poly-pharmacy) and trimethoprim-sulphamethoxazole in patients whose HIV tests are confirmed positive may have a survival advantage.

No significant difference was noted in the proportions of patients with positive blood cultures when the two groups were compared. This finding is unusual as bacteraemic pneumonia is reported to be commoner in HIV disease (Noskin et al, 1996; Koulla-Shiro et al, 1996). Again, sample sizes may be an important factor.

Streptococcus pneumoniae was the commonest organism cultured, accounting for 59 % of isolates. This finding is consistent with results obtained elsewhere (Polsky et al, 1986; Witt et al, 1987; Mandell et al, 2000). An uncharacteristic finding was the isolation of *Staphylococcus aureus* in 22 % of positive blood cultures. This may be explained by the high HIV infection rate of 76 % in this study. It is prudent to empirically cover for *Staphylococcus aureus* in severely ill HIV infected patients with CAP to avoid delays in commencing therapy.

Nine of the 37 patients (24 %) with clinically significant blood cultures required a change in antibiotic therapy. These were patients in who *Staphylococcus aureus* (7), *Eschericia coli* (1) and *Cryptococcus neoformans* (1) were isolated. Thus, blood cultures impacted on

antibiotic treatment one in four times when they were positive. There were no changes in therapy in all patients who had streptococcal bacteraemia. The practice in our centre is to administer 2.5 million units of penicillin G 6 hourly intravenously. Although this is explained by the initial administration of a second generation cephalosporin in most cases, clinically significant streptococcal resistance to penicillin is not a problem in our institution. This knowledge has come from local surveillance within Durban. High grade penicillin resistance to pneumococcus is less than 1 %. Intermediate grade resistance is less than 25 % but there is good clinical response to the relatively high penicillin doses used (Dr Peer, microbiologist, Lancet laboratories, Durban; personal communication). It is not routine to do penicillin sensitivity testing in our institution (for reasons just stated) and this study did not address that issue. This is in contrast to reports from other parts of the world (Waterer et al, 1999). Two subjects with *Staphylococcus aureus* pneumonia did not have a change in antibiotic therapy as they improved clinically on amoxicillin-clavulanic acid and a second generation cephalosporin. One of the two later died of an unrelated cardiac condition.

In summary, the yield from blood cultures in this study was high. Positive blood cultures in hospitalized patients with CAP have a crucial role in subsequent antibiotic switching and targeted therapy. Unexpected non-bacterial pathogens such as fungi may be cultured in HIV infected subjects.

CHAPTER 9

**The value of PCT in distinguishing CAP due to common
bacteria, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii***

9.1 Summary

Aims of Study

To evaluate the utility of procalcitonin in distinguishing CAP due to common bacteria, *mycobacterium tuberculosis* and *pneumocystis jirovecii* in a high prevalence setting where atypical presentations of CAP confound empiric clinical diagnoses

Methods

Two hundred and sixty six patients with CAP were investigated using a standard clinical algorithm. Serum samples for PCT levels were collected on admission. The PCT levels were determined by an ILMA assay using a kit supplied by BRAHMS (Appendix 9).

Results

A microbiologic diagnosis for CAP was obtained in 169 out of the 266 patients. The isolates comprised 44 cases of *Mycobacterium tuberculosis*, 31 of *Pneumocystis jirovecii* and 35 patients with bacterial pneumonia; 42 consisted of coinfections with *Mycobacterium tuberculosis*, gram positive and gram negative bacteria, *Pneumocystis jirovecii* and atypical organisms. The mean (standard error of the mean) PCT concentrations in the three groups were: *Mycobacterium tuberculosis* 4.16 (1.197) ng/ml; *Pneumocystis jirovecii* 1.138 (0.2911) ng/ml; bacterial pneumonia 19.48 (5.64) ng/ml; ($p < 0.0004$). The lower and upper 95 % confidence intervals were 1.749 ng/ml and 6.579 ng/ml for tuberculosis; 0.543 ng/ml and 1.734 ng/ml for *Pneumocystis jirovecii*; 8.021 ng/ml and 30.938 ng/ml for bacterial pneumonia.

Conclusion

Serum concentrations of PCT differ significantly in patients with CAP due to *Mycobacterium tuberculosis*, *Pneumocystis jirovecii* and common bacteria. The diagnostic ranges did not overlap. PCT may be important in distinguishing *Mycobacterium tuberculosis* and PCP in a high HIV prevalence setting where atypical presentations of pulmonary tuberculosis and PCP often confound the empiric clinical diagnosis.

9.2 Introduction

Procalcitonin is the propeptide of the hormone calcitonin. This propeptide is markedly elevated in septic burns patients (Reinhart et al, 2000; Bohuon, 2000). It is now established as a new marker of the inflammatory response to infection. The organ of production of this 13 kDA polypeptide is unknown but neuroendocrine cells that produce PCT have been found in the liver and lung tissue. The stimuli for its release are endotoxin, TNF- α , exotoxin and as yet unidentified costimulatory factors.

Studies of PCT have focused on its utility in discriminating infectious from noninfectious causes of inflammation (Reinhart et al, 2000). Levels correlate positively with the severity of septic shock (Cheval et al, 2000) and with severity of illness in melioidosis (Brunkhorst et al, 2000). In healthy subjects, PCT is not detectable and the levels are only moderately elevated in non-septic inflammatory processes, various metastatic cancers and with minor infection (Rau et al, 2000). Little or no elevation of PCT is found in viral infections (including HIV) but levels increase early during systemic bacterial and fungal infections and decrease when the infection resolves.

The value of PCT in distinguishing CAP due to *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, and common bacteria has not been previously studied. Sub-Saharan Africa is currently the epicenter of the HIV pandemic and the region has experienced a marked increase in HIV related pulmonary tuberculosis, bacterial pneumonia and PCP.

9.3 Patients and Methods

Two hundred and sixty six patients with CAP were recruited. The inclusion and exclusion criteria were as outlined in chapter 2. Serum for PCT concentrations was obtained on admission before the results of microbiology tests were known to the investigators. The recruitment period was March 2000 to October 2001. This was designed to enable the recruitment of patients from the PCP oropharyngeal washes arm of the study. Details of microbiology tests appear in Chapter 2. Procalcitonin measurements are detailed in appendix 9. Patients were stratified according to the organism (s) isolated on microbiology tests.

Data analysis

The PCT levels were correlated with the aetiology of the CAP. A One-way Analysis of Variance (ANOVA), Bartlett's test for homogeneity of variances and the Tukey-Kramer Multiple Comparisons test were used to analyze the data. Only single isolates without coinfection were considered in the final analysis although data on the remainder is provided.

9.4 Results

The aetiology of CAP was determined in 169 out of the 266 patients (63.5 %). One hundred and ninety eight (83 %) of the 238 patients who consented to HIV testing were positive. PCT was determined in the 266 patients without prior knowledge of the aetiology. No organisms were isolated in 97 subjects. The breakdown of the isolates were: *Mycobacterium tuberculosis* 44, gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) 35, *Pneumocystis jirovecii* 31, *Mycoplasma pneumoniae* 12,

Chlamydia pneumoniae 4, *Legionella pneumophila* 1 and the remaining 42 (24.8 % of isolates) cases were polymicrobial permutations of *Mycobacterium tuberculosis*, gram negative organisms, gram positive organisms, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*.

The mean PCT levels for *Mycobacterium tuberculosis*, common bacteria and *pneumocystis jirovecii* were 4.164ng/ml; 19.479ng/ml and 1.138ng/ml respectively (Fig 9.1 and 9.2). These differences were highly significant ($p < 0.0004$). The respective standard errors of the mean were 1.197 ng/ml; 5.640 ng/ml and 0.291 ng/ml. There was a significant difference between the mean PCT level for PTB and that for PCP (two tailed $p < 0.0435$). The mean PCT for pulmonary tuberculosis and bacterial pneumonia and PCP and bacterial pneumonia were significantly different ($p < 0.002$ and $p < 0.001$ respectively). Table 9.1 shows the lower and upper 95% confidence intervals for PCT. These were: PTB 1.749 - 6.579 ng/ml; common bacteria 8.021 - 30.938 ng/ml and PCP 0.543 -1.734 ng/ml.

PCT levels in atypical bacteria and other combinations

The mean PCT levels in 97 patients in which no organisms were isolated was 17.669 ng/ml. Seven subjects were coinfectd with both mycobacteria and bacteria. The mean PCT in this group was 18.8457 ng/ml. There were 12 and 4 patients infected with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* respectively. The mean PCT levels were 1.32 ng/ml and 1.61 ng/ml. PCT was 0.52 ng/ml in the patient who had *Legionella pneumophila*.

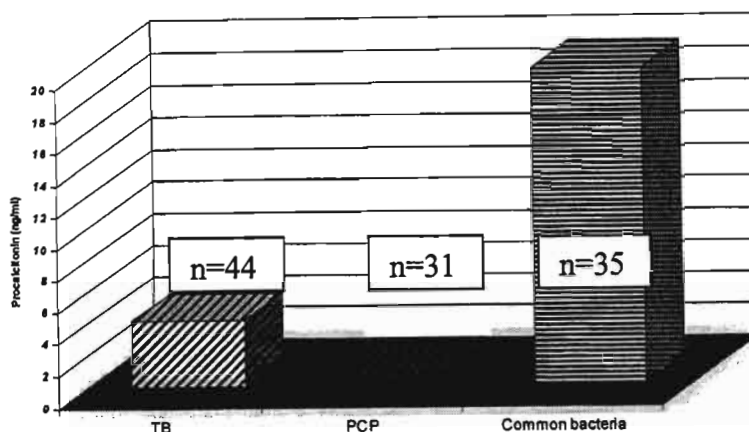


Figure 9.1 Mean PCT for PTB, PCP and common bacteria

Table 9.1 Comparison of procalcitonin levels

	TB	Common bacteria	PCP
Mean	4.164	19.479	1.138
SD	7.939	33.842	1.595
SEM	1.197	5.640	0.291
Median	0.930	6.105	0.387
Lower 95% CI	1.749	8.021	0.543
Upper 95% CI	6.579	30.938	1.734
Diagnostic Range	1.749-6.579	8.021-30.938	0.543-1.734

The two-tailed p values = 0.0435 (significant) for TB vs PCP; $p < 0.002$ for PTB vs bacteria; $p < 0.001$ for PCP vs bacteria. The one way analysis of variance (ANOVA) p value for all 3 is 0.0004, considered extremely significant.

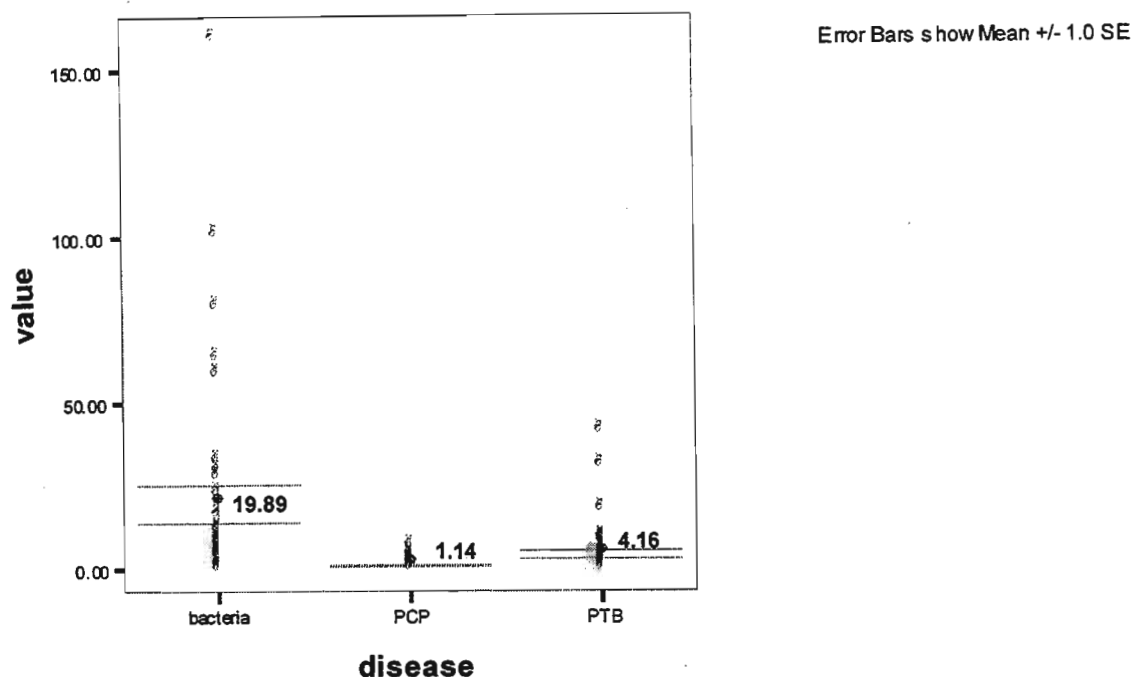


Figure 9.2 Scatter plots of PCT

9.5 Discussion

This study has shown for the first time that PCT may help to discriminate PTB and PCP from other causes of CAP in hospitalized patients in a high HIV prevalence setting. Mean PCT was 19 times higher in bacterial pneumonia compared to PCP and nearly 5 times the mean value in PTB ($p < 0.0004$). Analysis of data obtained for PCP and PTB alone showed a significant difference in the means ($p < 0.0435$).

Approximately 3.5 million new infections with HIV occurred in sub-Saharan Africa in 2001 bringing to 28.5 million the total number of people living with HIV/AIDS in the region (Joint United Nations Programme on HIV/AIDS, 2002). PTB is the commonest AIDS defining illness in HIV infected subjects in sub-Saharan Africa while bacterial

pneumonia is a common cause of morbidity and mortality in HIV disease (Cordero et al, 2000). The incidence of PCP, previously said to be rare in Africa, is on the increase with as many as 20% of patients with pneumonia having or coinfecting with *Pneumocystis jirovecii* in our unit. This study has shown a prevalence of 11% (chapter 4).

The differential diagnosis for HIV related CAP is wide and many patients may be too ill to be candidates for invasive or even semi-invasive procedures such as bronchoscopy or sputum induction. It is frequently not possible to determine the aetiology of CAP based on the history, examination and radiological findings alone. There is therefore a need for quick, non-invasive tests to aid the diagnostic algorithm.

The number of patients who were infected with atypical organisms was considered too small to be incorporated into the data analysis. However, it should be noted that mean serum PCT levels were 1.32 ng/ml and 1.61 ng/ml in mycoplasma and chlamydia infections respectively and thus may be difficult to differentiate, on the basis of this test alone, from PCP. Previous studies of CAP in South Africa have shown the proportion of cases of CAP due to mycoplasma and chlamydia to be variable (Maartens et al, 1994; Potgieter, 1995) but low compared to *Streptococcus pneumoniae*, which still remains the commonest cause of bacterial CAP worldwide (Mandell, 1995; Bartlett et al, 1998; Ruiz et al, 1999). It should be noted that in the 7 cases in which there was coinfection with *mycobacterium tuberculosis* and bacterial pathogens, the mean PCT was 18.8457ng/ml, a level that reflects bacterial infection. A similar conclusion can be drawn regarding the high mean of 17.669ng/ml in the 97 patients in whom no organisms were isolated. Thus a potential disadvantage of PCT measurement is the difficulty in interpreting the results if one is not sure of the number of pathogens in a patient.

The cascade of inflammatory cytokines that are released during systemic infection may determine the rate and intensity of PCT synthesis and release, thus accounting for the differences seen in the levels of this polypeptide. The number of microorganisms that enter the systemic circulation is also important and this is probably more with bacterial pneumonia compared to *Mycobacteria tuberculosis* and *Pneumocystis jirovecii* infection.

The half life of PCT is 24 hours. This is not altered during renal failure when compared to patients with normal renal function. There is no correlation with creatinine clearance, age or gender (Meisner et al, 2000). The pharmacokinetic properties of PCT enable it to serve as an early as well as late marker of infection and inflammation because the molecule is chemically stable in plasma and is not converted to calcitonin. This makes determination of PCT in the differential diagnosis of CAP due to common bacteria, *Mycobacterium tuberculosis*, and *Pneumocystis jirovecii* a particularly attractive test.

Two PCT tests are currently available. There is a PCT-QUICK which is a semi-quantitative test that takes 30 minutes to give a result. Its reading range is between 0.5 ng/ml and 10 ng/ml and can be done in the laboratory at any hospital. For the diagnostic ranges that this study has yielded this may be all that is required as the PCT-QUANTITATIVE test takes 2 hours to perform, requires more sophisticated laboratory work and costs more. It has a reading range of 0.5 - 1 000ng/ml. In addition to further studies, the issues of availability and cost will need to be addressed before the test is put to clinical use.

In conclusion, this study has demonstrated that serum concentrations of PCT differ significantly in patients with CAP due to *Mycobacterium tuberculosis*, *Pneumocystis*

jirovecii and common bacteria. There was no overlap in the diagnostic ranges. These findings may be important in the differential diagnosis of CAP and could be incorporated into the diagnostic and management algorithm. Further studies are required to verify these finding

CHAPTER 10

Summary and future directions

Sub-Saharan Africa is currently the epicentre of the HIV pandemic. In 2003, the WHO estimated the number of people living with HIV/AIDS in the region at 29.4 million people. The epidemic has not yet plateaued in this region. Pulmonary infections, in particular tuberculosis and common bacterial pneumonia, are common manifestation of HIV/AIDS. Community acquired pneumonia due to bacteria and *Mycobacterium tuberculosis* occurs at all levels of CD4 counts. There is a lack of important data on CAP in the era of HIV in Southern Africa. Knowledge is lacking on aetiology of CAP, coinfections, PCP and whether *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila* are common causes of CAP.

Pulmonary infiltrates in HIV infected patients are a serious diagnostic dilemma. It is difficult to distinguish PCP, bacterial pneumonia and pulmonary tuberculosis on clinical grounds alone. Patients may be too ill to be subjected to invasive diagnostic procedures such as bronchoalveolar lavage or lung biopsy. BAL is a safe procedure in stable patients but the expertise may not be available. It has diagnostic benefits and not necessarily therapeutic (removal of inflammatory plugs). Oropharyngeal washes and PCT, a new marker of the inflammatory response in infection, may be potential solutions to some of these diagnostic dilemmas. The role of routine blood cultures in patients with CAP is controversial and requires further clarification.

It is important to identify patients with CAP at risk of death and in need of hospitalization. The Fine Score is a pneumonia risk classification that is widely accepted but has not been validated in HIV infected patients with CAP.

Mortality data for Southern Africa in the era of HIV disease is lacking. The South African Thoracic Society guidelines for the treatment of CAP have not been validated. Physician compliance with the guidelines is unknown. There is no data on whether the guidelines have impacted positively on length of hospital stay and mortality.

Methods

The study was approved by the Ethics Committee of the University of KwaZulu-Natal. It was performed at King Edward VIII hospital, Durban from May 2000 to October 2001. Patients 18 years and above with CAP were studied. On admission, demographic and clinical data were collected. The Fine Score was calculated using a point scoring chart.

A standard algorithm for investigations was followed. Chest radiographs were obtained in all patients. Expecterated and/or induced sputum samples and venous blood samples were collected. Sputum samples were submitted for Gram stain and culture, Ziehl Neelsen stain and culture for AAFBs and *Legionella pneumophila* immunofluorescence. Pneumocystis immunofluorescence of induced sputum was performed in a subgroup of 95 consecutive patients with CAP. Anaerobic and aerobic blood cultures were performed prior to commencing antibiotics. Urine samples for legionella and streptococcus urinary antigen tests were refrigerated and later analysed in batches.

Serum samples were stored at -85°C and were then used for measurement of PCT. An immunoluminometric assay was used for PCT concentrations. Serology for *Chlamydia pneumoniae* was performed using an IgM measuring solid phase enzyme immunoassay. An ELISA kit was used to measure *Mycoplasma pneumoniae* IgM levels.

Fibre-optic bronchoscopy with BAL and transbronchial biopsy was performed in a cohort of patients with clinical and radiological features suggestive of PCP. Standard Haematoxylin and Eosin stains as well as Gomori, PAS, Gram and Ziehl Neelsen stains were performed on histology specimens. Oropharyngeal washes were performed in this group. HIV negative patients who had fibre-optic bronchoscopy for other diagnoses were used as controls. A Body Fluid Spin Protocol was used to extract DNA. PCR for *Pneumocystis jirovecii* was performed using a nested method. The method uses primers from the gene encoding the large mitochondrial ribosomal RNA.

The admitting team made the choice of empiric antibiotic treatment for CAP. Data on initial empiric antibiotic treatment, length of hospital stay and outcome were extracted from patient records.

Results

1. Aetiology of CAP.

Organisms were isolated in 222 out of the 430 patients studied. Eighty one percent were HIV infected. 255 pathogens were isolated. The most common organisms were *Mycobacterium tuberculosis* (39.6%), *Streptococcus pneumoniae* (34.5%) and *Staphylococcus aureus* (7.5%). Polymicrobial infections occurred in 16.2%. The commonest combination was *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*. Patients with coinfections were two and a half times more likely to die than patients without.

Two hundred and eighty two patients had serology for atypical bacteria. The most common organisms isolated in this cohort were *Mycobacterium tuberculosis* (34.4%),

Streptococcus pneumoniae (25.9%), *Mycoplasma pneumoniae* (17%), *Chlamydia pneumoniae* (4.9%) and *Staphylococcus aureus* (3.6%).

2. Incidence of PCP

Nine out of 95 (11%) consecutive patients with CAP had PCP. Six out of the 9 had coinfections. Mean CD4 count was 91cells/mm³.

3. Oropharyngeal PCR for *Pneumocystis jirovecii*

PCR in oropharyngeal washes had a sensitivity of 44% and a specificity of 79% when compared to histology from transbronchial biopsies. When compared to BAL, the sensitivity was 40% and specificity 77%.

4. SATS CAP guidelines

In patients with severe CAP, only 8% (14/182) of patients had antibiotic treatment prescribed according to recommendations in the guidelines. There was no significant difference in length of stay between the two groups (14 vs 12 days; $p = 1.0000$; odds ratio 1.167; 95% CI 0.3926 - 3.467) or in mortality (28.5% vs 19%; $p = 0.3549$; odds ratio 1.667; 95% CI 0.667 -4.161)

5. Fine Score pneumonia severity index:

Two hundred and eighty nine patients with bacterial CAP were analysed. The mortality rates per risk class were 13% and 20.6% for Grade II; 19% and 50% for Grade III; 38.9% and 42% for Grade IV; 100% and 100% in the HIV and non-HIV infected cohorts respectively. The mortality rate correlated positively with the Fine Score in both the HIV and non-HIV infected cohorts ($p < 0.0001$). There was no significant difference in mortality between the two groups ($p = 0.8766$).

6. Blood cultures

Forty-three of 181 patients (24%) had positive blood cultures. Thirty-seven patients (20.4%) had clinically significant isolates. The commonest organism was *Streptococcus pneumoniae* followed by *Staphylococcus aureus*. Antibiotic changes necessitated by a positive blood culture were made in 24% of patients. There was no correlation between yield from blood cultures and the severity of CAP as measured by the Fine Score.

7. Usefulness of PCT in the diagnosis of CAP:

Organisms were isolated in 169/266 patients. Data from patients with single isolates of common bacteria, *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* were analysed. The mean (standard error of the mean) PCT concentrations in the three groups were: *Mycobacterium tuberculosis* 4.16 (1.197) ng/ml; *Pneumocystis jirovecii* 1.138 (0.2911) ng/ml; common bacterial pneumonia 19.48 (5.64) ng/ml; ($p < 0.0004$). The lower and upper 95% confidence intervals were 1.749 ng/ml and 6.579 ng/ml respectively for tuberculosis; 0.543 ng/ml and 1.734 ng/ml respectively for *Pneumocystis jirovecii* and 8.021 ng/ml and 30.938 ng/ml for bacterial pneumonia.

Discussion and conclusions

1. This study provides evidence of the increasing incidence of *Mycobacterium tuberculosis* in the aetiology of CAP in both HIV and non-HIV infected patients. It was the commonest pathogen isolated in both cohorts. This is a dramatic change considering that less than 6% of cases of CAP were attributable to *Mycobacterium tuberculosis* before

the HIV epidemic. Although *Streptococcus pneumoniae* was the commonest bacterial cause of CAP, pulmonary tuberculosis should be excluded in all patients presenting with CAP. Coinfections occurred in 16% of patients and the mortality was significantly higher in this cohort. This finding is important in patients who show partial response to treatment or who continue to deteriorate despite appropriate antibiotic treatment. Although the sensitivity and specificity of serology for diagnosing CAP due to atypical organisms is low, the results of this study suggest that *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are not uncommon causes of CAP. Antibiotic cover for these organisms should be included in local treatment protocols. An important finding is the high ranking of *Staphylococcus aureus* as a cause of CAP. It has ranked low in all major studies published to date. The lack of search for viruses is a weakness in the study. Obtaining a history of prior antibiotic use would have been ideal but is difficult and unreliable in most patients seen at our institution.

2. Pneumocystis pneumonia should be considered as an important differential in African patients with CAP. One in ten HIV infected patients with CAP may have PCP. In patients with PCP, co-infections are common. A larger sample size would have provided more accurate data on the incidence of PCP. Simpler diagnostic laboratory tests are needed.
3. PCR on oropharyngeal washes for *Pneumocystis jirovecii* may have an important role to play in the diagnosis of PCP if the sensitivity and specificity can be improved.
4. Adherence to the SATS CAP antibiotic guidelines had no effect on length of stay or mortality. However, this conclusion may not be correct as the number of patients in which

the guidelines were followed was small (14/182 patients or 8%). Guidelines are not being adhered to in 92% of patients treated for severe CAP. The reasons may be multifactorial including lack of awareness, physician autonomy, economic considerations and an absence of institutional policy. A follow up study looking at the reasons for non-compliance with SATS guidelines and the effect of institutional policy is warranted. The high incidence of tuberculosis, the emergence of PCP and the high mortality in this study requires a review of CAP management in our institution.

5. This study is the first to demonstrate the usefulness of the Fine score in HIV infected patients with CAP. The Fine Score can be utilised in a resource poor setting. Its use should be encouraged to stratify HIV infected patients with CAP into risk groups.

6. Blood cultures should be performed in hospitalized patients with CAP. Twenty percent of patients had clinically significant organisms isolated on blood cultures. This is a high yield when compared with results from published studies. Positive blood cultures impacted on treatment of 1 in 4 patients.

7. This study showed that PCT was higher in common bacterial pneumonia and low in TB and PCP making it a potentially useful test in a diagnostic algorithm. Further studies are required particularly regarding its usefulness in co-infections.

Future directions

“CAP is a dynamic infectious disease syndrome.” There is unlimited scope for research in this field. Future studies need to be relevant, but not necessarily complex or labour intensive.

1. Aetiology. Specific hypotheses will need to be formulated and tested. In 50% of patients with CAP, no cause is found. The role of viruses needs to be researched. The search for new pathogens should continue. Utilization of molecular methods to identify pathogens even after the use of antibiotics must be explored. Its unlikely, however, that new pathogens will be discovered. It may be wiser to focus on developing more sensitive diagnostic tests. It is more likely that the organisms that we already know about are the causes of pneumonia in these patients, rather than undiscovered pathogens. Research in the area of geographical variation in the aetiology of CAP is very relevant and should be encouraged. How rigorous diagnostic testing should be is a matter of debate. What is certain, however, is that complete diagnostic testing is not achievable for reasons of cost, labour, logistics and inconvenience to the patients.
2. Diagnostic tests. The diagnosis of CAP due to *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* is difficult. There are no gold standards to use. Research in this area must continue.

The role of urinary antigen tests requires further research. Kits are already available for the diagnosis of *Streptococcus pneumoniae* and *Legionella pneumophila*. It may be possible to develop similar tests for other bacteria. There is scope for research in the area of PCR and the role of PCT.

3. Radiography. The non specific nature of pulmonary infiltrates in CAP makes it unlikely that ground breaking research will occur. The role and utility of computed tomography should therefore not focus on aetiology but perhaps on other aspects of management. The role of radiography in identifying complications and obstruction must be researched.
4. Antibiotics. Research into antibiotic resistance should be accelerated. Studies should look at correlation of resistant strains to outcome. The role of non-drug treatment modalities must not be ignored. This includes oxygen therapy, physiotherapy, bronchodilators and fluid therapy.
5. Cost. The economic implications of guidelines need to be evaluated. Currently there is no data on the cost effectiveness of guidelines. The answer may come from future studies.
6. Fine Score. This needs to be modified to include cachexia and low albumin as markers of malnutrition.

CHAPTER 11

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Patient Information/ Consent Form

You have been admitted into hospital with pneumonia. We are carrying out a study to determine which germs are causing this problem in our patients admitted to hospital, how bad this illness is in those admitted and what factors can assist us in predicting which patients will have severe disease. Your participation in this study will assist with the future management of patients with the same illness as you have now. Your permission is required for participation in this study.

The standard treatment, investigations and care that is given to all patients with pneumonia will be made available to you. This will include routine investigations to check your kidney function, liver function. In addition, sputum, bloods and urine cultures will be taken. Your permission is also required to perform a blood test to see if you are infected with the AIDS virus (HIV test). The doctor and nurse will explain to you in detail all the issues relating to this test. You have the right to refuse to have this test done without any prejudice to your treatment at this hospital. You will participate in this study only if you have agreed to an HIV test and you will be notified of the result of your test confidentially as well as the implications of the result. Should you not be getting better and / or we cannot determine which organism is causing your pneumonia you will be asked to give written informed consent for bronchoscopy. We may ask you to breathe into a special machine containing oxygen and a little bit of salt to make you cough in order to produce sputum which we can send to the laboratory for analysis to determine the cause of your pneumonia (sputum induction). We would also like to ask you a few questions about your social background and your knowledge concerning the common illness called AIDS. You

are free to choose not to answer any questions which you are uncomfortable with. All the answers you give will be treated strictly confidentially. The information you give us will assist in educating the public about this disease. You will be followed up for at least one month from the time of your discharge. You are free to ask any questions and we appreciate your cooperation with this important study. If at any time you wish to withdraw from the study you will be free to do so, without fear of prejudice or withdrawal of treatment. Please be assured that all information given by you will be treated confidentially. We plan to publish the results of this study and it will not identify you in any way.

I have read and understand the content of this form. My questions have been answered. I voluntarily agree to participate in this study.

Signature:

Date:

Witness:

Date:

Investigator:

Date:

The consent form was translated into a Zulu version.

Fine Score

Assign the indicated points if condition present		Points	Points Assigned
AGE	male	Age in years	
	Female	Age in years -10	
Nursing Home Resident		+10	
Coexisting illnesses:	Neoplastic disease	+30	
	Chronic liver disease	+20	
	Congestive cardiac failure	+10	
	Stroke	+10	
	Chronic Renal Disease	+10	
Physical examination findings: Altered mental status		+20	
	Respiratory rate 30 or >/min	+20	
	Systolic blood pressure < 90	+20	
	Temperature <35 or >40 deg C	+15	
	Pulse > or equal 125/min	+10	
Laboratory/Radiographic findings: Arterial pH <7.35		+30	
	Urea >11 mmol/l	+20	
	Sodium < 130 mmol/l	+20	
	Glucose>14 mmol/l	+20	
	Haematocrit <30%	+10	
	PaO ₂ <60 mmHg	+10	
	Pleural effusion	+10	
		Total Score	Total Score
Assign Grade Based on Fine Score			Grade

< 70=Grade II; 71-90=Grade III; 91-130=Grade IV; > 130= Grade V

Appendix 3

DNA extraction

STEP

1. Pipette 20 microlitres QIAGEN Protease (or Proteanase K) into the bottom of a 1.5 ml microcentrifuge tube.
2. Add 200 microlitres sample to the microcentrifuge tube. Use up to 200 microlitres whole blood, plasma, buffy coat, or body fluids, or up to 5×10^6 lymphocytes in 200 microlitres PBS.
3. Add 200 microlitres Buffer AL to the sample. Mix by pulse-vortexing for 15 sec.
4. Incubate at 56 degrees for 10 min.
5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
6. Add 200 microlitres ethanol (96-100 %) to the sample, and mix again by pulse – vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
7. Carefully apply the mixture from step 6 to the Siam spin column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuge at 6000 x g for 1 min. Place the Siam spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
8. Carefully open the Siam spin column and add 500 microlitres Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min. Place the Siam spin column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.
9. Carefully open the Siam spin column and add 500 microlitres Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20000 x g for 3 min

continue directly with step 10, or to eliminate any chance of possible Buffer AW2 carryover, perform step 9a, and then continue with step 10.

9a (optional): Place the Siam spin column in a new 2 ml collection tube (not provided) and discard the collection tube with the filtrate. Centrifuge at full speed for 1 min.

10. Place the Siam spin column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the Siam spin column and add 200 microlitres Buffer AE or distilled water. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g for 1 min.

Appendix 4

Mycoplasma serology

Assay procedure

Step

1. Place the desired number of strips into a microwell frame. Allow six Control/Cutoff Calibrator determinations per run. A reagent blank should be run on each assay.
2. Dilute test sera, Cutoff Calibrator and Control sera 1:81 in Serum Diluent Plus.
3. To individual wells add 100 microlitres of diluted patient sera, Cutoff Calibrator and Control sera. Add 100 microlitres of Serum Diluent Plus to the reagent blank well. Check software and reader requirements of the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25° C) for 30 minutes + or – 2 minutes.
5. Aspirate or shake out liquid from all wells. Add 250 – 300 microlitres of diluted wash buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper towelling to remove all liquid. Repeat the wash procedure two times. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.
6. Add 100 microlitres conjugate to each well, including the reagent blank well.
7. Incubate each well 30 minutes + or – 2 minutes at room temperature (21° to 25° C).
8. Repeat wash as described in step 5.
9. Add 100 microlitres Chromogen/ Substrate solution to each well including reagent blank well.
10. Incubate each well 10 minutes + or – 2 minutes at room temperature (21 to 25 degrees C).
11. Stop reaction by addition of 100 microlitres Stop Solution following the same order Chromogen/Substrate addition, including reagent blank well. Mix contents of the wells.

Wait a minimum of 5 minutes and read. The plate may be held up to one hour after addition of the Stop Solution before reading.

12. The developed colour should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650.

Interpretation

ISR Value- Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen Optical Density Value by the Cutoff Calibrator Value determined in step 3.

ISR value	Results
< 0.90	Negative
0.91-1.09	Equivocal
> 1.10	Positive

Appendix 5

Assay procedure for *chlamydia pneumoniae*

Step I

1. Pipette 100 microlitres of transparent IgM removing reagent into microstrip
2. Pipette into IgM removing reagent 10 microlitres of diluted specimens and the ready for use controls into microstrip.
3. While pipetting, mix well and cover the microstrips with plastic sheet.
4. Incubate for 1 hour (+or – 5 mins) at 37⁰ C.
5. Empty the wells into a suitable biohazardous container or aspirate the well contents with a washer.
6. Add 300- 400 microlitres of washing solution into each well.
7. Empty the wells.
8. Repeat the washing cycle five times in total.

Step II

1. Pipette 100 microlitres of the conjugate into each well and cover the microstrips with plastic sheet.
2. Incubate for one hour at 37⁰ C.

Wash the wells five times in total as in items 5-8 in Step I.

Step III

1. Pipette 100 microlitres of the TMB-substrate solution into each well.
2. Incubate for 30 minutes at room temperature in a dark place.

Step IV

1. Stop the enzyme substrate reaction by adding 100 microlitres of stopping solution (0.45 M H₂SO₄) into each well.

Measure the absorbance immediately at 450nm.

The results are expressed in Signal/cut-off units (S/CO).

Use the formula for calculations:

$$\text{S/CO sample} = (\text{A sample} - \text{A blank}) / (\text{A cut off} - \text{A blank})$$

Interpretation of Results

S/CO < 0.5 = Negative

0.5 < S/CO < 1.1 = Equivocal

S/CO > 1.1 = Positive

Test procedure for PCT concentrations in serum

1. Preparations
 - Allow all kit components and patient samples to warm up to room temperature.
 - Reconstitute tracer
 - Agitate all liquid reagents, including patient sera, gently before use
 - Number the coated tubes
 - Prepare washing solution: dilute 11 ml concentrate with distilled water to yield 550 ml
 - Prepare the luminometer for use
2. Pipette 20 microlitres PCT standards of increasing concentrations into the tubes S1 a, b ...S6 a, b. Pipette 20 microlitres of each control into the tubes Ko1 a, b Ko2 a, b, and 20 microliters of each serum sample into the tubes P1, a, b etc.
3. Pipette 250 microlitres tracer into all test tubes.
4. Mix the tubes for a short period of time on a sample mixer to ensure homogeneity of the liquid. Cover the test tubes with adhesive foil and incubate them on a horizontal rotator (170-300 rpm) for 1 hour-1 hour 15 mins at room temperature (18-25⁰ C).
5. Add 1 ml of the washing solution to each tube prior to decanting the liquid off completely.
6. Add 1 ml of the washing solution four times to all test tubes and decant off the liquid completely after each washing step. After the last rinsing step, turn the tubes upside down and allow to drain for 5-10 mins on clean blotting paper.
7. Place all tubes in the luminometer in the order defined by the measuring protocol.
8. Start luminescence measurement with automatic injection of 300 microlitres LUMItest Basiskit reagents 1 and 2. Recommended measuring time is 1 second per tube.

9. Calculation of results.

Using the standard curve or the recalculated master curve; the measured luminescence signal values can then be used to directly determine the PCT concentration of the unknown samples in ng PCT/ml.

***Streptococcus pneumoniae* urinary antigen test**

Assay procedure

1. Bring patient urine to room temperature (15-30⁰ C) then swirl gently to mix.
2. Dip a Binax swab into the urine sample to be tested, completely covering the swab head.
3. There are two holes on the inner right panel of the device. Insert swab into the bottom hole (swab well). Firmly push upwards so that the swab tip is fully visible in the top hole.
4. Hold reagent A vertically above the device and slowly add three (3) free falling drops of Reagent A.
5. Immediately peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in 15 mins.

Interpretation of results

A negative sample will give a single pink to purple coloured Control Line in the top half of the window, indicating a presumptive negative result.

A positive sample will give two pink to purple coloured lines indicating antigen detection.

Specimens with low levels of antigen may give a faint patient line. If no lines are seen, or if just the Sample line is seen, the assay is invalid and should be repeated.

Appendix 8

***Legionella pneumophilla* urinary antigen test**

1. Bring patient urine to room temperature (15-30⁰ C).
2. Dip a Binax swab into the urine sample.
3. Insert swab into the bottom hole (swab well). Firmly push upwards so that the swab tip is fully visible in the top hole.
4. Hold Reagent A vertically and slowly add two (2) drops of Reagent A to the bottom hole.
5. Immediately peel off adhesive liner from the right edge of the test device. Close and securely seal the device. Read result in 15 mins.

Interpretation of Results

A negative sample will give a single pink to purple colored Control Line in the top half of the window indicating a presumptive negative result.

A positive sample will give two pink to purple coloured lines. This means that antigen was detected. Any visible line is positive.

If no lines are seen or if just the sample line is seen the assay is invalid and should be repeated.

Flowcytometry: Immunophenotyping by the lyse technique

1. Equipment

Automatic dispensing pipettes

3x3/8 Plastic tubes

Yoken marker

Pipette tips

Vortex

Flowcytometer with computer/printer

2. Reagents

(a) Isoflow (sheath fluid)

(b) Conjugated monoclonal antibodies:

1. M02RD1/KC56FITC

2. CONTROL IGG1RD1/FITC

3. CD4RD1/CD8FITC

4. CD3FITC/CD19RD1

3. Method

(a) Blood must be carefully mixed by inversion

(b) Adjust counts of working dilution to 1×10^6

(c) Place 100 microlitres of whole blood into respective tubes

(d) Add 10 microlitres of the respective monoclonal antibody

(e) Vortex samples

(f) Stand for 20 mins in the dark

(g) Sample is then ready to analyse on flow cytometer

- (h) The original blood sample (EDTA) is then submitted to the routine laboratory for processing through a haemocytometer (Advia or Max M), including differential.

Normal values

Absolute CD3 count – 800-2800

Absolute CD4 count – 550-1955

Absolute CD8 count – 250-1200

Blood cultures

Materials

Equipment

- (a) Bactec-alert automated blood culture system
- (b) Microscope
- (c) Incubators 37⁰ C
- (d) Electric slide warmer
- (e) Vortex mixer

Media

- (a) Bactec broth
- (b) Culture media

Reagents

- (a) Gram Stain

Procedure

- (a) Capture patient's data on computer
- (b) Removal of positive vials/bottles
 - 1. On cabinet door scan "remove positive"
 - 2. Remove bottles with illuminated red lights and scan bottle bar code only
- (c) Removal of negative blood culture bottles
 - 1. On the cabinet door scan remove negatives
 - 2. Remove all bottles with illuminated red lights
- (d) Processing of positive blood cultures

1. Venting of blood culture bottles: Cleanse the septum with a cotton- tipped swab soaked in 70 % alcohol. Insert the venting device through the septum
2. Prepare a Gram stain from the positive blood cultures as follows:
 - (i) Gently agitate the bottle and add one drop of sample onto an appropriately labelled slide.
 - (ii) Allow the preparation to dry on an electric warmer
 - (iii) Gram stain smears
3. Examine the Gram stains of positive cultures and record all results on work sheets
4. Subculture the samples onto media according to the morphology and Gram reaction of the organism

Gram stain

(a) Reagents

Crystal violet

Lugol's iodine

96 % Ethanol

10 % Carbol Fuchsin

Procedure

1. Heat fix slide
2. Pour crystal violet and let it stand for 1 minute
3. Rinse with distilled water
4. Pour Lugol's iodine and let it stand for 1 minute
5. Rinse with distilled water
6. Decolourize with acetone and rinse with distilled water
7. Pour 10 % carbol fuchsin and let it stand for 1 minute
8. Rinse it off with distilled water
9. Pat it dry on filter paper
10. Read under 100x magnification with oil on slide

Appendix 12

Ziehl-Neelsen stain for AAFBs

1. Flood slides with strong carbol fuchsin for 5-7 minutes. Heat slides evenly. Soak the swab in 70 % alcohol, ignite, and apply heat to the undersurface of each slide. Repeat this procedure after 3 mins.
2. Rinse slide in tap water
3. Decolourize with 3 % acid –alcohol for 2 mins. Thick smears can be left longer.
4. Rinse in tap water.
5. Counter stain with methylene blue for 30 seconds.
6. Rinse slides in tap water.
7. Allow to dry.

***Pneumocystis jirovecii* immunofluorescence**

Principle

The Shield Detect IF *pneumocystis jirovecii* kit is a qualitative indirect immunofluorescence kit for the detection in human bronchoalveolar lavage and induced sputum of *pneumocystis jirovecii* oocysts. The presence of *pneumocystis jirovecii* oocysts is indicative of infection by *pneumocystis jirovecii*. Pre-treated patient material is placed onto a microscope slide, dried, fixed and enzyme digested. A monoclonal antibody directed against *pneumocystis jirovecii* oocysts is then reacted for 5-15 minutes with specimen. Fluorescence isothiocyanate – conjugated antibody directed against the monoclonal antibody is then reacted with the specimen. Following rinsing, drying and mounting, the specimen is viewed using a fluorescence microscope. Positive specimens are characterized by readily identified characteristic fluorescent cysts.

Kit Components

1. Detect IF anti-*pneumocystis jirovecii* monoclonal antibody
2. Detect IF fluoresce in-isothiocyanate conjugated anti-mouse antibody
3. Detect IF enzyme
4. Dilute hydrochloric acid
5. Detect IF enzyme diluent
6. Detect IF *P. carinii* control slide
7. Detect IF patient specimen slides
8. Detect IF mounting medium

Procedure

Reconstitute lyophilised enzyme with 200 microliters of 0.001 M HCl. Allow to stand at room temperature for 10 mins.

1. Centrifuge sample. Wash the pelletable sample 2-3 times in distilled water.
2. Resuspend the final pellet by vortexing in a small amount of distilled water
3. Spread 10-20 microliters of resuspended pellet over the entire area of the patient's slide well. Evaporate to dryness at 37⁰ C.
4. Fix the specimen by overlaying with 1-2 drops of acetone. Allow to evaporate at room temperature.
5. Remove one positive control slide from the satchet and allow the condensed moisture to evaporate.
6. Rinse slides with a stream of distilled water and allow to air dry.
7. Prepare a sufficient volume of working strength enzyme by diluting the reconstituted enzyme 1:10 with enzyme diluent.
8. Overlay the test specimen with 20 microlitres of working strength enzyme and the positive control well with 5 microlitres of working strength enzyme.
9. Incubate slide for 30 mins in a humidified chamber at 37⁰ C.
10. Rinse slides with a stream of distilled water.
11. Air dry slides.
12. Add 15 microliters of FITC anti-mouse antibody to test sample.
5 microliters of FITC anti-mouse antibody to positive
Incubate at 37⁰ C for 5-15 mins in a humidified chamber.
13. Rinse the slides with a stream of distilled water and air dry.
14. Add 15 microlitres of FITC anti-mouse antibody to test sample.
5 microliters of FITC anti-mouse antibody to positive

Incubate at 37⁰ C for 5-15 mins in a humidified chamber.

15. Rinse slides well and air dry.
16. Place a drop of mounting medium onto each well in use and apply a cover slip.
17. Examine the control well at 100X and check that the oocysts are bright and clearly visible.
18. Examine the test specimen for the presence of bright to medium bright apple-green oocysts.

Interpretation

1. Test specimens containing 5 or more fluorescent oocysts over the whole slide are positive.
2. Test specimens containing 1-5 oocysts are scored as equivocal.
3. Samples containing no fluorescent oocysts are scored as negative.

Appendix 14

PCR

1. Make a total volume of 50 microliters as follows: 10 mM Tris HCl, pH 8.3, 50 mM KCl,
2. 5 mM MgCl₂, 200 microlitres dATP, dTTP, dCTP, dGTP (DNA Polymerize mix), 100 pmol of each primer and 1U of Taq DNA polymerase.
3. Add 50 microliters of mineral oil and 10 microliters of DNA extract through the mineral oil layer.
4. Denature at 94⁰ C for 30 seconds.
5. Anneal at 55⁰ C for 30 seconds.
6. Extend primer at 72⁰ C for 45 seconds.
7. Repeat steps 4-6 thirty-five times i.e. 35 cycles of amplification.
8. Remove 1.5 microliters of first PCR product and add into fresh reaction mixture containing second set of primers.
9. Amplify for a further 25 cycles.
10. Analyse amplified DNA products by electrophoresis in 2 % agarose gel in 0.5x TBE buffer by ethidium bromide staining.⁹⁹