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Factors Influencing Early and Late Mortality in Adults with Invasive Pneumococcal Disease in Calgary, Alberta

by

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Abstract

IPD continues to cause significant incidence of mortality and 60% of deaths occur within 5 days of presenting to hospital. Multinomial regression was performed to analyze 3 outcomes: early mortality (<5 days post-presentation), late mortality (5-30 days post-presentation), and survival.

Patients with severe IPD had increased risk of early and late death. In multinomial regression with survivors as baseline, the risk of early death in those with a Charlson index score ≥2 was 5.3X (1.5-18.8); the risk of late death in those with less severe disease was 6.4X (1.4-29.5). Patients who never received appropriate antibiotics had 3.5X (1.6-8.0) the risk of early death. Patients receiving appropriate antibiotics >48 hours after presentation had 4.7X (1.58-13.9) the risk of late death. Age was not statistically associated with risk of early or late death.

The primary analysis showed that severe IPD and multiple comorbidities increase the risk of early and late death, while age does not. Delay in receiving appropriate antibiotics increases the risk of death and may be a modifiable factor.

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List of Abbreviations and Nomenclature

Abbreviation	Definition
IPD	Invasive Pneumococcal Disease
CSF	Cerebrospinal fluid
CAP	Community Acquired Pneumonia
PPV-23	Pneumococcal Polysaccharide Vaccine (23 serotypes)
PCV7	Pneumococcal Conjugate Vaccine (7 serotypes)
PCV13	Pneumococcal Conjugate Vaccine (13 serotypes)
ICU	Intensive Care Unit
RR	Risk Ratio
RRR	Relative Risk Ratio
OR	Odds Ratio
TMP/SMX	Trimethoprim/Sulfamethoxazole
APS	Acute Physiology Score (APACHE without age and comorbidity calculation)
APACHE II	Acute Physiology and Chronic Health Evaluation Score II
PSI	Pneumonia Severity Index
CLSI	Clinical and Laboratory Standards Institute

Chapter One: Introduction

Streptococcus pneumoniae, is a significant pathogen worldwide, particularly in young children, the elderly and immunocompromised individuals. It is responsible for both invasive and non-invasive disease manifestations leading to substantial clinical and economic impact.[1]

Invasive pneumococcal disease (IPD) is defined as acute illness with isolation of *S. pneumoniae* from a normally sterile site, including blood, pleural fluid, or cerebrospinal fluid (CSF).[2, 3] Although invasive disease accounts for a small proportion of the overall disease burden caused by *S. pneumoniae*, it results in considerable morbidity and mortality, particularly among elderly adults.

Elderly adults aged 65 years and older are at the highest risk for mortality from IPD due to weakened immune systems and higher proportions of co-morbidities.[4] The elderly account for about one-third of all cases of IPD; however, they represent nearly half of the deaths.[1] Compared with children, the IPD case-fatality rate is higher in all adult age groups. In Calgary, Alberta, between 1998-2004 the IPD case-fatality rate among adults (≥16 years of age) was 13.3%, while the case-fatality rate for children (<16 years of age) was only 2.3%.[5]

The proportion of deaths that occur in the first 5 days after presentation with an invasive *S. pneumoniae* infection has not changed significantly since early in the antibiotic era. Unpublished data analysis from the Calgary Area *Streptococcus pneumoniae* Epidemiology Research (CASPER) study in Calgary, Alberta, described that the proportion of all deaths due to IPD that occurred in the first 4-5 days after presentation to a healthcare facility was 54% in 1998 – 2007,[6] which is similar to the proportion seen in the United States in 1952-1962 (60%).[7] Using a larger and more recent dataset from 2000 to 2009, this thesis research expanded on the preliminary CASPER study findings. The purpose of this thesis research project was to examine factors influencing death in adult patients within the first 5 days (early mortality) and death between 5 and 30 days after presentation with IPD (late mortality). The hypothesis for

this study was that those who die early from IPD present later in the course of their illness and, therefore, at a more severe disease stage. If this were the case, their illness would be less responsive to treatment because the infection is already well established, or they may present too late for treatment to be an option, so only palliative measures are taken. Identifying what demographic, clinical, and microbiological factors may be involved in early mortality and late mortality is a critical step in understanding the pathogenesis of *S. pneumoniae* and the disease progression of IPD. It may also guide understanding of what medically modifiable factors may help to further reduce case-fatality rates due to IPD in adults. While current research focuses on risk factors for overall mortality, few studies before this have closely examined factors involved in early mortality due to IPD in adults.

Chapter Two: Background

2.1 Microbiology and Laboratory Methods

2.1.1 Streptococcus pneumoniae: the Sugar-Coated Microbe

Streptococcus pneumoniae, commonly known as pneumococcus, was one of the first well-studied microbes. *S. pneumoniae* are gram positive, elongated cocci, and commonly exist as diplococci: two spherical cells joined together. They can also exist as single cells or short chains. Historically, Oswald Avery,[8] who did extensive research with *S. pneumoniae* in the early 1900's, referred to *S. pneumoniae* as the sugar-coated microbe due to its external polysaccharide capsule that acts as an important defence mechanism against the human immune system.

Streptococcus pneumoniae commonly colonizes the human nasopharynx without causing clinically apparent disease. However, all disease caused by *S. pneumoniae* is preceded by nasopharyngeal colonization by the disease causing strain.[9] Under the correct conditions *S. pneumoniae* can cause both non-invasive and invasive disease manifestations. Non-invasive manifestations include otitis media, sinusitis, and pneumonia, while invasive disease manifestations include bacteremia, meningitis, and bacteremic pneumonia. Cases of invasive pneumococcal disease (IPD) are less common than non-invasive cases, but are of greater concern due to higher morbidity and mortality.

2.1.2 Streptococcus pneumoniae Serotypes

More than 92 different *S. pneumoniae* serotypes have been characterized.[3, 10] Serotypes are distinguishable by the immunochemistry of their polysaccharide capsule, and pathogenicity varies with serotype.[3] *Streptococcus pneumoniae* serotypes have been grouped into 46 serogroups.[3, 10, 11] Serogroups can consist of multiple serotypes that are immunologically cross-reactive.[3] Cross-reactivity occurs when the host immune system

generates antibodies against one serotype that can recognize and bind other serotypes in the same serogroup.

Correlations have been found between invasive disease manifestations and the serotype of *S. pneumoniae*.[3, 11] For example, in a review by Hausdorff et al. the authors suggested that serogroup 1 was isolated from blood significantly more often than from the CSF in young children, older children, and adults, while serogroup 23 was more frequently isolated from the CSF than blood.[11] However, disease manifestations vary with age; therefore, it is difficult to draw conclusions regarding an association between serogroups and disease manifestation without considering age. The review by Hausdorff et al. attempted to address this issue by only considering serogroups that showed a tendency to be isolated from the same location in both children and adults.[11]

Age and co-morbid conditions may affect which serotype causes invasive disease.[12] For instance, a study involving 5 countries (Canada, USA, United Kingdom, Spain, Sweden) found that serotype 1 caused invasive disease largely in patients less than 65 year of age, while serotype 23F caused invasive disease more often in people over 65 years of age.[13] Sjostrom et al. found that serotypes 1 and 7F were the most invasive, tending to act as primary pathogens and cause disease in otherwise healthy people.[13] Serotypes 3, 6A, 6B, 8, 19F, and 23F behaved as opportunistic pathogens, usually causing invasive disease in people with co-morbidities.[13]

Associations between serotype and outcome have also been shown. A variety of epidemiological and experimental studies have indicated that serotypes in serogroup 9, as well as serotypes 3 and 11A are more likely to cause severe disease.[7, 12, 14, 15] Serotypes defined as being less invasive and opportunistic, because of their increased likelihood of also causing asymptomatic nasopharyngeal colonization, appear to be associated with increased risk of mortality when they do cause invasive disease.[13, 16] In addition, compared with a single reference serotype, other serotypes have been shown to be more or less likely to be associated with mortality. For example, Harboe et al. showed that in individuals 5 years and older, serotypes 31, 11A, 35F, 17F, 3, 16F, 19F, 15B, and 10A

were associated with increased mortality compared to the reference group, serotype 1.[12] Similarly, Henriques et al. found serotype 3, 6B and 19F to be associated with increased mortality compared to reference serotypes 1 and 7F.[17] A meta-analysis by Weinberger et al. suggested patients infected with serotypes 3, 6A, 6B, 9N and 19F were at greater risk of death compared to patients infected with serotype 14, while patients infected with serotypes 1, 7F and 8 were at decreased risk of death.[18] The microbiological mechanisms that cause certain serotypes to be more virulent are not fully understood. There is also evidence that serotype has a greater influence on outcome in the case of bacteremia than meningitis.[12] For meningitis, host factors rather than serotype had a greater impact on outcome.[12]

In the Netherlands, Jansen et al. showed that serogroups known to have high potential for invasive disease in children tended to affect relatively healthy adults and cause milder disease manifestations, whereas more fragile adults, such as the elderly, were often infected by serotypes with lower invasive potential.[19] The elderly have weakened immune systems due to aging, as well as increased proportions of co-morbidities, thus providing opportunity for less invasive, opportunistic pathogens to cause disease.[3, 13] Similarly, Lujan et al. showed an association between risk of death and less invasive, opportunistic serotypes (3, 6A, 6B, 8, 19F).[16] This association held up in a multivariable analysis adjusted for Pneumonia Severity Index class V, Charlson score, Age < 60 versus ≥ 60, alcohol abuse, and American Thoracic Society/Infectious Disease Society of America criteria (ATS/IDSA).[16] In this study, the ATS/IDSA criteria[20] were used to assess severity of pneumonia.[16] CAP was considered severe in those cases that met 1 of the two major criteria, or 3 of the 9 minor criteria.[16, 20]

Although some serotypes are more likely than other serotypes to be associated with fatal IPD cases, it is not clear whether the serotype of a particular *S. pneumoniae* strain is more important than host patient factors such as underlying co-morbid conditions or disease severity. Two studies that used multivariable analysis to determine the role of individual serotypes (compared with individual baseline index serotypes, either serotype

1 or 14) as well as patient comorbidity and disease severity, found that IPD caused by some serotypes were more likely to be found in fatal cases than a particular index serotype.[12, 18] However, other studies that conducted multivariable analysis of the relative importance of any of multiple serotypes, as well as host comorbidity and disease severity, found that specific serotypes, or groups of serotypes, were either not significantly associated with fatal cases, compared with host factors,[21] or that only some groups of serotypes were significant in addition to host factors.[16] Therefore, it is apparent that some individual serotypes are more or less likely to be associated with fatal IPD cases than other individual serotypes; however, when IPD cases caused by multiple serotypes are considered together as a group, host factors are generally more important than serotype in fatal cases.

Occasionally outbreaks by serotypes that are not common causes of invasive disease may occur in a particular population. An outbreak of invasive serotype 5 infections occurred in an impoverished, urban population in Calgary, Alberta between 2006 and 2007 as well as a serotype 8 outbreak in 2005.[22] In August 2006, an outbreak of invasive infections by serotype 5 occurred in a similar population in Vancouver, British Columbia.[23] Historically, in Canada, serotype 5 has not been a commonly recovered serotype.[23]

2.1.3 Human Immune Response and Bacterial Immune Evasion

The complement protein system constitutes part of the innate human immune system. This cascade of proteins is activated through recognition of a pathogen and results in either opsonization and phagocytosis of the pathogen, or formation of a membrane attack complex protein.[24] This system is an important part of immune defence against *S. pneumoniae*. However, *S. pneumoniae* has developed methods of immune evasion that allow it to continue to cause both mild and severe disease.

The best understood factor in *S. pneumoniae* immune evasion is the external polysaccharide capsule, which is also the factor that distinguishes serotypes. The

polysaccharide capsule assists *S. pneumonia* in evasion of the immune system by interfering with activation of the complement cascade. The complement cascade is a system of proteins and glycoproteins that interact to form functional immune complexes.[24] There are three pathways to activation of the complement system: classical pathway, alternative pathway, and lectin pathway. The classical pathway is activated by binding of C1q protein to the Fc portion of an antibody that is bound to antigens on the bacterial cell, or when C1q binds directly to the bacterial cell surface.[25] The alternative and lectin pathways do not require antibodies bound to the pathogen surface.[25] Each pathway results in formation of the C3 convertase which is cleaved into C3a and C3b proteins.[25] The final outcome is either opsonization and phagocytosis of the bacterial cell, or formation of the membrane attack complex, which forms a protein channel in the membrane of the target pathogen cell, causing cell lysis.[24, 25] The *S. pneumoniae* capsule may reduce the amount of C3b that can bind to *S. pneumoniae* as well as interfering with interaction of receptors on host phagocytes with C3b that is deposited on the bacterial cell.[24, 26] This helps to prevent phagocytosis of the bacterial cell, which involves an immune cell (phagocyte) engulfing and breaking down the bacteria.

Recent evidence suggests that certain serotypes of *S. pneumonia* may be capable of binding the C4BP complement protein.[25] C4BP is an inhibitor of the classical complement pathway, and binding of this protein is a known method of complement evasion used by other bacteria such as *Haemophilus influenzae* and *Moraxella catarrhalis*.[25] However, binding of the C4BP protein was shown to be serotype specific with serotype 4, 6B, and 7F exhibiting intermediate binding and serotype 14 exhibiting strong binding of C4BP.[25]

Other virulence factors produced by *S. pneumoniae* include pneumolysin (a cytotoxin) and pneumococcal surface protein, PspC. Pneumolysin acts to inhibit the antimicrobial activity of polymorphonuclear leukocytes (phagocytes) by causing complement activation away from the *S .pneumoniae* cell surface.[27] This activation away from the cell prevents deposition of C3b on the bacterial cell surface, which therefore inhibits

opsonization and prevents the classical complement cascade from being completed.[27] The PspC protein family is involved in translocation of *S. pneumonia* into nasopharyngeal epithelial cells[28] and can inhibit the alternative complement pathway from being activated by binding the host complement inhibitor factor H.[29, 30] *S. pneumoniae* virulence factors are still not fully understood and research continues in this area.

Human antibody response allows clearance and instils immunity, but only against the infecting serotype; therefore, a person can be re-infected with other serotypes.[24] The polysaccharide capsules that distinguish serotypes are immunogenic. Vaccination can be an effective method of prevention due to the ability of the antibody response to recognize and clear infection by a serotype that has been previously encountered by the immune system through immunization.

2.1.4 Laboratory Tests to Isolate S. pneumoniae from Clinical Specimens

The standard method for distinguishing *S. pneumoniae* from other disease causing organisms is through colony morphology, alpha haemolysis tests, optochin screening, and bile solubility reactions. *S. pneumoniae* is sensitive to optochin about 95% of the time (i.e., will not grow in the presence of optochin). Sensitivity is indicated by a zone of no bacterial growth $\geq$ 14 mm around the optochin disk.[31] *S. pneumoniae* have a unique morphology on a gram-stain, appearing as gram-positive, lancet-shaped diplococci. On agar, *S. pneumoniae* grow as glistening colonies, about 1mm in diameter. When grown on blood agar, *S. pneumoniae* produce a zone of alpha hemolysis, which appears as a transparent green zone around the colony. Bile solubility is a key reaction that distinguishes *S. pneumoniae* from other streptococcus species. Bile will selectively lyse colonies of *S. pneumoniae*, while other alpha-hemolytic Streptococcus species are resistant to the activity of bile.

2.1.5 Methods of Serotyping

There are several methods available for serotyping *S. pneumonia*. The Quellung reaction is the classical method, first described by German scientist Fred Neufeld in 1902.[32] This reaction involves one at a time combination of the bacterial sample with separate anti-sera that specifically recognize each serotype.[33] A positive result is indicated by the appearance of cellular swelling due to binding of the specific antibody with the *S. pneumoniae* capsule.[33] Although generally highly specific, there are some downfalls to this method: some cross-reactivity has been observed between serotypes, the interpretation of results is subjective, the process is tedious, and the anti-sera are expensive.[34, 35] As a result, molecular techniques have been developed that may eventually replace the Quellung method.[33]

Molecular techniques largely involve the use of polymerase chain reaction (PCR). Culture results can be insensitive, and culturing of the organism is necessary for the Quellung reaction.[36] The use of PCR eliminates the need for culturing, allowing for more rapid serotyping of respiratory specimens, which could be useful for surveillance purposes.[36] There is also evidence to suggest that PCR-based methods are more sensitive than the standard Quellung reaction.[35] PCR methods involve serotype specific primers that target genes unique to the serotype that the primer is made to detect.[34] The primers allow for amplification of the serotype specific sequences that bind to the primers.[34] The PCR products are then detected directly or by gel electrophoresis.[34] The genes targeted by the primers can be determined by migration patterns allowing for differentiation of the serotype present in the sample.

2.2 Invasive Pneumococcal Disease

Invasive pneumococcal disease (IPD) is preceded by nasopharyngeal colonization by the invasive serotype of *S. pneumoniae*.[37] IPD is defined as acute illness with isolation of *S. pneumoniae* from a normally sterile site, including blood, pleural fluid, and cerebrospinal fluid.[2, 3] Invasion of the blood commonly occurs prior to invasion of other sites.

2.2.1 Bacteremia

Commonly, the presentation of *S. pneumoniae* bacteremia is associated with a focal infection in an organ site such as the lungs or brain. However, in some cases, bacteremia without focus can occur. The presence of bacteremia in patients with pneumococcal pneumonia suggests a worse prognosis.[38]

Bacteremia is defined as the presence of bacteria in the blood and may be transient, intermittent, or continuous. Transient bacteremia, where bacteria are only in the blood for a brief time, is common and of less concern, while continuous bacteremia is more serious. Occasionally, bacteremia may progress to cause a more serious infection resulting in sepsis, severe sepsis, or septic shock. All-cause bacteremia without focus has been shown to have poorer prognosis than bacteremia with a known source.[38-40]

2.2.2 Pneumonia

Pneumonia is an infection of one or both lungs. *Streptococcus pneumoniae* is the most common etiologic agent of community acquired pneumonia (CAP), and is a less common cause of hospital-acquired pneumonia.[41] CAP is the most common invasive presentation of *S. pneumoniae,* especially among elderly adults. Non-invasive pneumonia occurs when *S. pneumoniae* spreads from the nasopharynx to the lungs, usually through aspiration. Invasive pneumonia with bacteremia may occur after aspiration to the lungs

and invasion to the bloodstream or after initial invasion to the bloodstream and focal spread to the lungs and/or pleural fluid.[37]

2.2.3 Empyema

Empyema is infection of the pleural space: the space between the lung and the chest wall. Infection of the pleural space generally occurs due to spread of infection from the lung. Empyema is a complication of pneumonia caused by *S. pneumoniae*.[37]

2.2.4 Meningitis

Meningitis is an infection of the meninges: membranes that envelope the central nervous system. Meningitis is the most severe form of IPD with a mortality rate ranging from 13% to 57%.[42-45] Mortality due to pneumococcal meningitis is higher in adults than children,[42, 43] and *S. pneumonia* is the most common cause of community-acquired bacterial meningitis in adults.[46] Presence of an underlying comorbidity is a risk factor for developing meningitis due to *S. pneumoniae*.[43]

2.2.5 Endocarditis

Endocarditis is infection of the lining of the heart chambers and heart valves. *Streptococcus pneumoniae* is an uncommon cause of bacterial endocarditis, accounting for 1-3% of cases since the introduction of penicillin.[47] Alcohol abuse is suggested to be one of the main risk factors for pneumococcal endocarditis, but the reason for this is unknown.[47] Typically endocarditis due to *S. pneumoniae* follows pneumonia in alcoholic adults and meningitis may be an additional complication.[47]

2.2.6 Peritonitis

Peritonitis occurs when infection spreads to the peritoneum, the thin lining of tissue around the inner wall of the abdomen. Inflammation caused by infection of the peritoneum results in pain and tenderness of the abdomen. This is a less common manifestation of *S. pneumonia* infection.[48, 49] In Alberta from 2000-2005 just over 1% of IPD cases had peritonitis.[48] Predisposing factors for peritonitis may include alcohol abuse and liver cirrhosis.[48, 49] Peritonitis may be preceded by or concurrent with a respiratory infection, or the peritoneum may be the primary infection site accompanied by bacteremia.[48] In Alberta in 2000-2005 primary peritonitis occurred in 39% of cases and secondary peritonitis in 52% of cases. Nine percent of cases appeared to be an ascending infection through the genital tract in young girls.[48]

2.2.7 Septic Arthritis

Septic arthritis is inflammation of a joint due to an infectious agent. *S. pneumonia* can infect the normally sterile joint fluid to cause septic arthritis: an uncommon presentation of IPD.[50] Septic arthritis by *S. pneumoniae* is commonly not associated with another site of manifestation, including bacteremia, in which case the infection may occur due to seeding of the joint during transient *S. pneumoniae* bacteremia from a mucous membrane source.[51]

The most common joint affected is the knee, followed by the shoulder.[52] In a review of 190 cases of septic arthritis due to *S. pneumoniae* the case fatality rate was reported to be 19% in adults.[51] The presence of pneumococcal bacteremia was shown to be a strong predictor of mortality and bacteremia was more common in adults with septic arthritis due to *S. pneumoniae* than other organisms.[51]

2.3 Treatment and Prevention of IPD

2.3.1 Antibiotic Treatment and Resistance

Historically, *S. pneumoniae* was susceptible to penicillin and other beta-lactam antibiotics. However, the presence of penicillin resistant *S. pneumoniae* has been increasing.[53, 54] In 2008 the Clinical Laboratory Standards Institute changed the definition of resistance for Penicillin, Cetriaxone, Cefotaxime and Cefepime so that there are separate minimum inhibitory concentration (MIC) cut-offs for meningitis and non-meningitis.[55] A higher dose of antibiotics is required to cross the blood-brain barrier and achieve a high enough MIC in the CSF to treat meningitis due to *S. pneumoniae*. The previous cut-offs reflected the MIC required for an isolate to be considered susceptible for treatment of meningitis, but these cut-offs were more liberal than necessary for non-meningitis.[56] A lower dose of antibiotics is sufficient to achieve an appropriate MIC in the blood or pleural fluid. Previously the MIC values for both meningitis and non-meningitis were equivalent to the current meningitis cut-offs.[56] The new guidelines allow for an isolate with a higher MIC in vitro to still be considered susceptible in non-meningitis cases.[55] This change in MIC cut-offs has contributed to an apparent decline in rates of beta-lactam resistance.

Some studies have suggested that combination treatment for *S. pneumoniae* infections have improved outcomes.[57-59] Most commonly this is a combination of a ß-lactam with a macrolide. Other studies have suggested no improvement with dual therapy.[60-62]

2.3.2 Vaccination

Current research supports the importance of an effective prevention such as vaccination to decrease mortality due to IPD.[63, 64] The issue of antibiotic resistance complicates treatment of IPD and emphasizes the importance of prevention. Multidrug-resistant *S. pneumoniae* are becoming a greater problem and vaccination is an effective method of reducing the incidence of IPD while also reducing the use of antimicrobials.[63-65]

Currently there are two kinds of vaccines available in Canada: polyvalent plain polysaccharide vaccines, and polyvalent protein-polysaccharide conjugate vaccines.

2.3.2.1 Polysaccharide Vaccine

While some form of polysaccharide vaccine has been available since the 1920s, the current 23-valent pneumococcal polysaccharide vaccine (PPV-23) was developed in the 1960's and 1970's.[1] The 23-valent vaccine is composed of purified polysaccharides from the outer capsules of 23 pneumococcal strains that are most commonly isolated from adults in Europe and the United States (serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F).[3]

The PPV-23 vaccine stimulates the formation of opsonizing antibodies, which will interact with the pathogen if it is introduced into the immunized host and will facilitate phagocytosis of the pathogen. Evidence of the effectiveness of PPV-23 is inconsistent. Some studies have shown PPV-23 to be clinically effective in elderly and high-risk adults,[64] and evaluations indicate that vaccination of adults older than 65-years of age is a cost-effective method to prevent IPD in the elderly.[1] Breiman et al. found the PPV-23 to be effective at instilling immunity in adults infected with human immunodeficiency virus (HIV).[66] However, a randomized controlled trial of the PPV-23 vaccine in non-immunocompromised middle aged and elderly adults found that PPV-23 failed to prevent pneumonia.[67] Other studies suggest that PPV-23 may be effective in healthy adults, but significantly less effective in immunocompromised adults and other groups such as the Navajo people.[68, 69] A study in Edmonton, Alberta showed that in a population at high risk of recurrent pneumonia the PPV-23 vaccine was ineffective in preventing death or subsequent hospitalization with pneumonia, meningitis, sepsis or similar infection within 5 years; however, this particular study did not confirm the microbiological cause of the repeat infection.[70]

There is also evidence that vaccine effectiveness may decrease with increasing age.[68] Furthermore, the vaccine appears to have low effectiveness in developing countries. In

Uganda, French et al. showed the PPV-23 vaccine to be ineffective at preventing pneumococcal disease in those with HIV.[71] Vaccines may be less effective for people with immune disorders because they are unable to develop a protective immune response.

2.3.2.2 PCV7 vaccine

Polysaccharide vaccines such as the PPV-23 are insufficiently immunogenic in children due to their inability to stimulate a T-cell dependent response.[2, 3, 63] As a result, efforts were made to develop a vaccine in which polysaccharides from pneumococcal capsules are covalently linked to carrier proteins to establish a vaccine that is immunogenic in children, and more immunogenic in adults.[3] The protein-conjugate vaccines stimulate a more robust T-cell dependent response, compared to the T-cell independent response from the polysaccharide vaccine.[72] The 7-valent pneumococcal conjugate vaccine (PCV7) was developed containing polysaccharides from the 7 most common serotypes that cause IPD in children in the USA and Canada conjugated to carrier proteins (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F).[3] The PCV7 vaccine was introduced for routine use in children in the USA in 2000 and was introduced in Canada and other countries in 2002.[73] In a randomized, double-blind trial of nearly 38,000 children in 23 different centers in California, the 7-valent pneumococcal conjugate vaccine was shown to be effective in reducing the risk of pneumonia in young children.[63] Furthermore, a Cochrane review concluded the PCV vaccines to be effective at reducing disease caused by the serotypes contained in the vaccine.[74] Vaccination of young children with the PCV7 vaccine has also resulted in a decrease in the incidence of pneumococcal disease in older children and adults, particularly in high-risk and elderly adults (>65 years).[72, 73, 75] This effect is due to herd immunity: the chain of infection is broken because diseased people less frequently encounter and infect susceptible people.

In addition, while the PPV-23 vaccine seems to be unsuccessful at instilling immunity in HIV infected Ugandan adults, the PCV7 vaccine appears to be effective at providing

protective immunity in this group of people.[76] The PCV7 vaccine was only introduced in Canada in 2002; therefore, we do not yet know whether it will be successful at producing long-term immunity.

Following introduction of the PCV7 vaccine there was a decrease in colonization and disease by the *S. pneumoniae* serotypes that are included in the PCV7 vaccine,[63, 65, 77] and a corresponding increase in colonization and disease by non-vaccine strains.[78-80] Strains of *S. pneumoniae* have been found to compete in human colonization,[81] and when the competition from vaccine strains was removed there was opportunity for colonization by non-vaccine strains. However, a review of the evidence suggests PCV vaccines to be effective in terms of reducing overall incidence of disease caused by *S. pneumoniae* despite serotype replacement.[74]

2.3.2.3 PCV13 vaccine

In July 2010 the PCV13 conjugate vaccine was licensed in Canada. This vaccine contains 6 more serotypes (serotypes 1, 3, 5, 6A, 7F and 19A) as well as the same seven present in the PCV7.[82] The PCV13 includes serotype 19A, which has been one of the major replacement serotypes since the introduction of the PCV7 vaccine.[82] There is also a 10 valent conjugate vaccine that was licensed in Canada in 2009. However, since this vaccine does not contain some of the most prevalent current serotypes causing IPD, most notably 19A, it has been superseded across Canada by PCV13. The 10-valent pneumococcal conjugate vaccine includes the same 7 serotypes as PCV7 as well as serotypes 1, 5, and 7.

The introduction of vaccines has altered the epidemiology of invasive *S. pneumoniae* infections, including the prevalence of vaccine serotypes. Most invasive infections now occur in people with co-morbidities.[42] The PCV7 vaccine has also altered the distribution of serotypes causing the majority of colonization, which precedes invasive disease.[37] Non-vaccine serotypes have replaced vaccine serotypes as the primary colonizers of the nasopharynx.[37] Although some non-vaccine serotype replacement has occurred in

invasive disease, so far it has not made up for the overall decline in invasive disease following the implementation of PCV7 vaccination in developed countries.[37]

2.4 Epidemiology and Risk Factors for IPD

2.4.1 Age

IPD is most common in the very young and the very old.[73, 83, 84] Several studies have shown mortality rates from IPD to be highest in the elderly due to higher numbers of comorbidities and decreased immune function.[72, 85, 86]

Following the introduction of the PCV7 vaccine, the incidence of IPD due to PCV7 serotypes in adults over 50 years of age decreased from 22.4 to 10.1 cases per 100,000 people.[72] However, the PCV7 vaccine consisted primarily of serotypes that commonly cause disease in children, while adults are commonly infected by a number of non-vaccine serotypes including 22F and 11A.

2.4.2 Gender

Men have been shown to be at greater risk for IPD.[84, 87] However, it has been suggested that this is due to higher rates of alcohol abuse among men.[84] When alcoholics were excluded by Burman et al. they found the difference between male and female adults nearly disappeared.[84] Although other studies suggest that when stratified for smoking and alcohol abuse the male-female difference does not fully equalize.[88]

2.4.3 Alcohol Abuse

Alcohol is among the most commonly abused substances in Western cultures, and is associated with decreased overall health.[89, 90] Excessive alcohol consumption has been linked to increased susceptibility of a host to infectious diseases, including pneumococcal

pneumonia.[90, 91] Rates of IPD among adults who abuse alcohol has been reported to be 11 times higher than rates in healthy adults.[92] A study in the Netherlands showed that alcoholic patients were more likely than non-alcoholics to have meningitis caused by *S. pneumoniae* as opposed to another pathogen.[89] Alcoholics were also at greater risk of developing systemic complications from the meningitis; however, this study did not find a significant difference in mortality rates due to meningitis between alcoholics and non-alcoholics.[89]

2.4.4 Cigarette Smoking

There are a limited number of studies that focus on cigarette smoking as a risk factor for IPD. However, one case-control study showed that cigarette smoking was associated with increased odds of IPD among otherwise healthy, non-elderly adults.[93] This study found that a greater proportion of cases smoked than controls.[93] They also found evidence of a dose-response relationship: odds increased with increasing number of pack-years of smoking.[93] The biological mechanism associated with increased risk of IPD with exposure to tobacco smoke is poorly understood; but damage to the respiratory tract may be involved.[93] Pneumonia may be a more common manifestation among smokers due to the effect of smoking on mechanical immune mechanisms such as mucus production and ciliary clearing.

2.4.5 Ethnic Distribution

Some ethnic groups are at higher risk of IPD. Indian populations like the White Mountain Apache have been found to have higher rates of IPD compared to the general population.[94] People of African-American descent have also been shown to have increased risk of IPD.[84, 95] However, it has been suggested that this may be due to higher rates of poverty and HIV.[96]

2.4.6 Crowding

Crowding is a common factor involved in epidemics of infectious diseases spread through droplet and contact. Pneumococcal epidemics are uncommon, but when they do occur it is usually in a situation that involves crowding such as homeless shelters.[23, 88] Similarly, crowding through hospitalization and institutionalization may increase risk of IPD in elderly populations by providing opportunity for colonization by *S. pneumoniae.*

2.4.7 Co-morbidities

Invasive pneumococcal disease occurs more frequently in adults with co-morbidities such as central nervous system diseases, heart disease, pulmonary disease, malignancies, renal failure, diabetes, and immune disorders.[42, 86, 92, 97] The incidence of IPD in healthy adults is about 8.8 cases of IPD per 100,000 healthy adults.[92] The incidence rates for adults with co-morbidities is much higher at 46.2/100,000 persons with diabetes and up to 503.1/100,000 persons with immunocompromising conditions such as HIV.[92]

2.4.7.1.1 HIV

Human immunodeficiency virus (HIV) infections inhibit the body's ability to mount an immune response against other invading organisms. As a result, HIV infection is perhaps the greatest risk factor for acquiring an invasive pneumococcal infection. Jordano et al. estimate that those with HIV have 60 times the risk of IPD compared to those without a known HIV infection.[98] In this study, the authors found that rates of other opportunistic infections had decreased in the era of Highly Active Antiretroviral Therapy (HAART) for patients infected with HIV; however, the rate of IPD remained unchanged.[98] Similarly, Redd et al. found HIV patients to have 100 times the risk of IPD compared to the general

population.[99] These authors suggest that the effect of HIV to dampen humoral immunity may play a role in the increased risk of IPD rather than the effect of HIV on T cells.[99] Humoral immunity (antibody response) plays an important role in the body's defence against *S. pneumoniae*.[99] Although HIV infected patients are at higher risk for IPD, there is limited evidence of efficacy of the PPV-23 vaccine in this population.[66, 71, 100]

Frankel et al. found penicillin non-susceptibility was more prevalent in HIV positive patients.[101] However, the same was found in immunocompromised patients without HIV, suggesting it is the condition of being immunocompromised, not HIV specifically, that may be associated with increased penicillin resistance.[102] It is likely that the increased use of antibiotics in these populations is what drives the increased resistance.

2.4.7.1.2 Pulmonary Disease

Pulmonary disease is a clinically relevant risk factor for IPD. Pulmonary disease damages the lungs, resulting in decreased ability to clear infections. Pneumonia especially is a common complication of pulmonary diseases. In Finland, the rate of IPD in patients with chronic pulmonary disease is 34.3 per 100,000.[103] In the USA in 1999-2000 the incidence of IPD in patients with chronic pulmonary disease was 62.9 cases per 100,000 people while the incidence in healthy adults was approximately 8.8 cases per 100,000 people.[92]

2.4.7.1.3 Cardiac Disease

Patients with cardiac disease are at increased risk for infections due to weakened heart muscles, which can cause decreased movement of blood throughout the body. This may result in opportunity for bacteria to attach to heart valves and causes the body to be less effective at transporting immune cells and oxygen to sites of infection. In the USA, the incidence of IPD in patients with cardiac disease was 93.7 per 100,000 in 1999-2000

compared to 8.8 per 100,000 in healthy adults.[92] In Finland, the incidence of IPD in patients with cardiac failure was 47.1 per 100,000 from 1995-2002.

2.4.7.1.4 Malignancy

Underlying malignancy is a recognized risk factor for IPD. A 2005 study in the USA found that adults with a haematological malignancy had 38.3 times the risk of IPD compared to healthy adults.[92] Adults with a solid organ malignancy were shown to have 22.9 times the risk of IPD compared to healthy adults in the same study.[92] An Alberta study in 2010 showed an increase in risk of IPD among adults with malignancies compared to the general population.[104] This study reported unadjusted odds ratio suggesting adults with multiple myeloma have 62.8 times the risk of malignancy compared to the general population. Lung cancer increased the risk of IPD by 13.4 times, chronic lymphocytic leukemia increased risk by 12.6 times, acute myeloid leukemia or acute lymphoblastic leukemia increased risk by 11.9 times, and Hodgkin's and non-Hodgkin's lymphoma increased risk by 4.4 and 5.8 respectively compared to the general population.[104]

2.4.7.1.5 Diabetes

Diabetes may be a risk factor for infectious diseases due to decreased immunity. In particular, diabetics may be at increased risk of pneumonia due to hyperglycemia, increased risk of aspiration, impaired lung function, and pulmonary microangiopathy.[105-107] Diabetics also commonly have other co-existing morbidities that increase susceptibility to infection.[107] Although there is no convincing evidence at this time that diabetics are at increased risk for IPD, they do seem to have greater morbidity and mortality once an infection occurs.

2.4.7.1.6 Splenectomy

Patients who have undergone a splenectomy have been reported to have 12 times the risk of infection compared to the general population.[108] In a study of overwhelming infection in asplenic patients, *S. pneumoniae* caused 87% of the infections.[109]

2.5 Epidemiology of IPD in Calgary, Alberta

Pneumococcal disease is widespread in Calgary, although it has changed with the introduction of the polysaccharide conjugate vaccine (PCV7) for children.[5] The rate of disease caused by serotypes in the PCV7 vaccine and related serotypes declined by 93.4% in 2004 compared to 1998 and 2001.[5]

Surveillance from 1998-2007 through the CASPER study showed an overall 30-day case-fatality rate of 10%.[73] The age-specific 30-day case-fatality rates for children were low, including 0% in the 0- to 5-month-old group and the 5- to15-year-old group.[73] The highest case-fatality rate in children was in the 2- to 4-year-old group at 6%.[73]

Thirty-day case-fatality rates for adults aged 16-64 years was 8%, while in older adults the case-fatality rate increased to 20% in adults aged 65-84 years and to 24% in adults 85 years and older.[73]

Meningitis was a common manifestation of IPD in children in Calgary from 1998-2007 particularly those aged 0-5 months.[73] In older adults pneumonia was more common.[73]

Since the introduction of the PCV7 vaccine in Calgary in 2002, there has been a continued decline in the incidence of IPD due to PCV7 serotypes, and an increase in the incidence of IPD due to non-PCV7 serotypes.[73] In particular, serotype 19A has become a problem due to increased incidence and high rates of antibiotic resistance.

2.6 Mortality Risk Factors in Patients with IPD

A literature search was performed on March 23, 2011 to ensure a thorough overview of risk factors for mortality due to IPD was obtained. The search was run in PubMed with the terms "*Streptococcus pneumoniae*," "mortality," and "risk factors," combined with AND. Only studies examining IPD in adults were included. Non-English language studies were excluded. The papers from the literature search and the risk factors for mortality from IPD are summarized in Appendix A. The following is a summary of the main risk factors.

2.6.1 Age and Gender

Age-related changes occur in both the innate and adaptive arms of the immune system, which can result in decreased ability to fight infection.[110] However, the most important factors involved in increased risk of IPD and death in elderly adults is the incidence of chronic comorbidities that result in an increased susceptibility to infections and decreased ability to overcome infections. The elderly account for about one-third of all cases of IPD; however, they represent nearly half of the deaths.[1] In West Virginia in 1999, the case fatality rate for pneumococcal meningitis was 36.8% in all adults and 45.8% in adults older than 50 years.[42] The case-fatality rate for community-acquired pneumonia is 10-20% and approaches 35% in the elderly.[86, 111]

Gender may also be associated with mortality in IPD patients in some age groups. A Finnish study found that in the 18-49 year old age group, both men and women have similar case fatality proportions; however, in the 50-64 year old age group men had higher case fatality than women.[103]

2.6.2 Comorbidities

Patients with comorbidities are at increased risk of death due to IPD. Many comorbidities cause decreased immune function resulting in increased risk of mortality due to infections.

Despite the increased risk of IPD and infection with penicillin resistant *S. pneumoniae,* there is no clear increase in mortality due to IPD in HIV positive patients.[101, 112] Though, interestingly, Grau et al. found that the case-fatality in HIV patients with IPD increased from before the HAART era to after the HAART era.[112] Perhaps because HIV patients are surviving long enough to develop IPD and to have a greater number of other comorbidities.

A Spanish study provided evidence that patients with non-AIDS immunocompromising conditions are at increased risk of mortality due to IPD.[102]

2.6.3 Alcohol Abuse

There is little evidence to suggest alcoholism alters the risk of mortality due to invasive pneumococcal disease, though it may be associated with increased severity. Alcoholics have been shown to have increased risk of developing systemic complications from meningitis.[89] The same study did not find a significant difference in mortality rates between alcoholics and non-alcoholics.[89] Similarly, a European study found an independent association between alcoholism and community-acquired pneumonia caused by *S. pneumoniae;* however, they did not find any difference in mortality between alcoholics, ex-alcoholics, and non-alcoholics.[113] This study did conclude that alcoholics tend to present with more severe cases of IPD.[113] Afessa et al. also did not find increased risk of mortality among alcoholics in a largely African-American population; however, this was a univariable analysis only.[114]

2.6.4 Antibiotic Susceptibility

So far, most studies have not found a significant difference in the risk of mortality with disease caused by penicillin-resistant *S. pneumoniae* and disease caused by penicillin-susceptible *S. pneumoniae*.[14, 86, 111, 115-117] Feikin et al. examined the presence of antibiotic resistance among cases of IPD in parts of California, Georgia, Maryland, Tennessee, and Toronto, Ontario. Their results showed no significant association between age-adjusted case-fatality rates and infection with antibiotic resistant *S. pneumoniae*; however, they did find a statistically significant association between death after the fourth hospital day and resistant *S. pneumoniae*.[86] They suggested that mortality in the first 4 days may be associated more with illness severity as opposed to therapeutic interventions.[86] Feikin et al. concluded that although antibiotic resistance is an issue, the most important risk factors for mortality due to IPD continue to be old age and co-morbidities.[86] Similarly, an international, observational study by Yu et al. assessing the clinical impact of antibiotic resistance in *S. pneumoniae* determined that antibiotic resistance in *S. pneumoniae* does not yet require changes to clinical treatment of pneumococcal pneumonia.[118] They did not find a difference in mortality between those patients receiving an antibiotic that the isolated strain of *S. pneumoniae* was sensitive to in vitro, and those receiving an antibiotic that was inactive in vitro against the isolated strain of *S. pneumoniae*.[118] An international study involving 5 countries also showed that penicillin resistance did not impact patient outcome.[13]

However, the relationship between penicillin non-susceptible *S. pneumoniae* (PNSP) and death remains controversial. A review by Telyjah et al., suggested that patients infected with PNSP are at increased risk of death.[119]

2.7 Early Mortality in Patients with IPD

Mortality due to IPD can be influenced by factors such as patient age, co-morbidities, and *S. pneumoniae* serotype. Many studies have investigated risk factors for overall mortality

due to IPD. However, to our knowledge, there are few studies investigating the factors involved in early versus late mortality due to IPD.

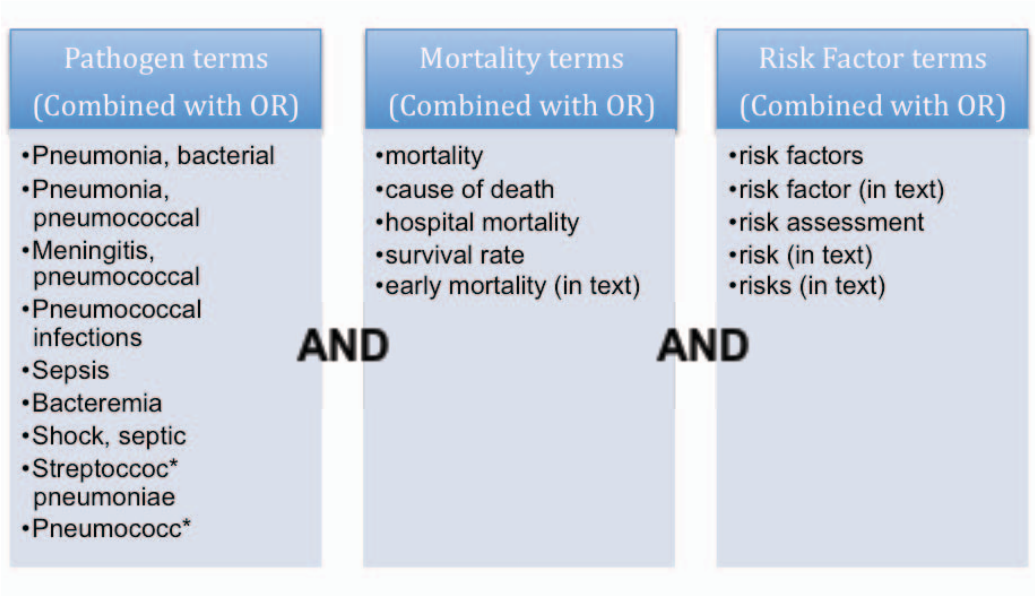
A study published in 1964 by Austrian and Gold showed that among patients who died of IPD, 60% died in the first 5 days after presentation with the invasive infection.[7] For the first 5 days after onset of IPD, the case-fatality rate in adults was similar for penicillin-treated patients, serum therapy-treated patients, and untreated patients.[7] After this 5-day period the case-fatality rate for penicillin-treated patients declined, showing penicillin to be effective.[7]

In 2000, Feikin et al found that 9% of patients with fatal cases died on the day of hospital admission and 21% died the following day.[86] Only 4% of the deaths occurred after 30 days of hospitalization.[86] In Calgary, Alberta, unpublished data analysis suggests that although the number of deaths has decreased, the case-fatality rate in the first five days in Calgary in 1998-2007 (54%) is similar to the case-fatality rate in Brooklyn in 1952-1962 (60%).[6, 7] Advances in treatment and vaccination have not altered the proportion of deaths that occur in the first five days. This raises questions about whether there is anything medically modifiable in patients that die early.

2.7.1 Literature Review

A literature search was performed July 26, 2010 in an attempt to capture all English language papers published in the past 25 years that pertain to early mortality due to IPD. It is difficult to differentiate early mortality papers from mortality papers, as there is no MeSH term for early mortality. However, a search in MEDLINE was performed using the search terms indicated in Figure 1 in an attempt to best capture early mortality papers. This search method brought up 755 references. The titles and abstracts were screened for relevance to early mortality due to IPD. Six papers were found to be relevant to the proposed study outlined here, but none of them duplicated it. The paper by Marrie et al. was the most similar to the research in this thesis, but was published and found after the initial search was completed. The relevant papers are outlined in Table 1.

Figure 1. Search method for early mortality risk factors literature search



Search Key:

- (*) indicates any ending acceptable
- (in text) indicates that search engine was instructed to search titles and abstracts for this term
- All terms that do not have (in text) next to them are MeSH terms.

Table 1. Early Mortality Literature Search Results

Study	Study focus	Study Groups	Analysis Type	Predictors of Early Mortality	Risk Measure (95% CI)	P-value
Marrie et al. (2011)	Invasive pneumococcal disease	Patients with IPD who survived, patients with IPD who died, and patients with IPD who died within 5 days of presentation	Multivariable survival analysis: Hazard ratio	Age 18-40 years (protective)	0.49 (0.27-0.88)	0.02
				Mechanical ventilation	2.13 (1.32-3.43)	0.002
				Altered mental status	5.42 (3.13-9.39)	<0.0001
				Cardiac arrest	2.71 (1.56-4.72)	0.0004
				Polysaccharide vaccine serotypes	0.51 (0.31-0.83)	0.007
				High mortality serotype compared to low/no mortality serotype	4.74 (1.40-16.07)	0.01

Study	Study focus	Study Groups	Analysis Type	Predictors of Early Mortality	Risk Measure (95% CI)	P-value
				Single antibiotic	2.36 (1.46-3.82)	0.0005
				2 concurrent antibiotics	0.30 (0.15-0.58)	0.0004
				>2 concurrent antibiotics	0.11 (0.03-0.46)	0.002
Blanco et al. (2008)	All-cause sepsis		Univariable analysis OR (95%CI)	Haemotological failure (based on Sequential Organ Failure Assessment score (SOFA))	1.5 (1.3-3.4)	NR
				Liver failure (based on SOFA score day 1)	2.0 (1.6-6.3)	NR

Study	Study focus	Study Groups	Analysis Type	Predictors of Early Mortality	Risk Measure (95% CI)	P-value
				Acquisition of infection prior to ICU admission	2.2 (1.0-4.4)	NR
				Logistic organ dysfunction score	1.2 (1.1-1.4)	NR
Garcia-Vidal et al. (2008)	All-cause community acquired pneumonia	Death in ≤48 compared to survivors and deaths after 48 hours	Logistic regression OR (95% CI)	Age ≥ 70	2.7 (1.4-5.3)	NR
				Altered mental status at admission	2.5 (1.3-4.8)	NR
				Shock at admission	7.6 (3.5-16.5)	NR
				Multilobar pneumonia	2.0 (1.0-3.8)	NR

Study	Study focus	Study Groups	Analysis Type	Predictors of Early Mortality	Risk Measure (95% CI)	P-value
				Discordant antibiotic therapy	11.3 (3.5-36.4)	NR
				Bacteremic pneumococcal pneumonia	2.4 (1.1-5.2)	NR
		Death in ≤48 hours compared to deaths after 48 hours	Logistic regression OR (95% CI)	Shock at admission	2.7 (1.0-7.1)	NR
Garau et al. (2007)	All-cause community acquired pneumonia	Early mortality (<2 days after admission)	Logistic regression OR (95% CI)	High pneumonia severity index (PSI) score (IV and V class)	13.0 (4.0-42.6)	<0.01

Study	Study focus	Study Groups	Analysis Type	Predictors of Early Mortality	Risk Measure (95% CI)	P-value
		*unclear whether early mortality compared with survivors or with survivors and late deaths		ICU admission	4.6 (2.1-9.9)	<0.01
				X-ray showing multi-lobar involvement	2.8 (1.4-5.5)	<0.01
Macias et al. (2004)	All-cause sepsis	Patients with severe sepsis who died compared to those who survived	Logistic Regression	Severe Protein C deficiency	<40% compared to approximately 48%	<0.05
				Elevated Interleukin-6	>8 compared to approximately 6.	<0.05

NR=Not reported

Six relevant papers were found in the literature search for early mortality due to IPD. Only the papers by Balakrishnan and Marrie et al. were IPD-specific.[120, 121] The other 4 looked at early mortality due to all-cause pneumonia, sepsis, or bacteremia.[122-125] Only multivariable results are reported in the table unless multivariable analysis was not done.

The three Spanish studies and the study from the UK identified early mortality as death in 48hrs or less.[121-124] The other two defined early mortality as less than 5 days.[125, 126]

Three of the studies were conducted in Spain. Spanish data may not be generalizable to Canada due to variations in healthcare systems as well as difference in climate. Invasive pneumococcal disease has been shown to have seasonal variation.[127]

The retrospective study by Balakrishnan et al. differed in its focus from the proposed study, as it does not compare early and late mortality. It focused on determining factors that can predict overall mortality early so that patients can be flagged as being at a high risk for death. It also focuses on only pneumococcal bacteremia rather than all forms of IPD. This paper is not outlined in the table as there were no relevant results for early mortality risk factors.

Two papers looked at all-cause mortality due to CAP.[122, 123] Although *S. pneumoniae* causes a large percentage of CAP, these studies do not consider pneumococcal disease specifically and focus on pneumonia rather than all invasive disease manifestations. Therefore, these results may not be generalizable to patients with IPD. In the study by Blanco et al. *S. pneumoniae* was identified in a small proportion of cases (21.2%), and the proportions did not add to 100%, suggesting that most patients had more than one microorganism identified in a sample.[124]

The only American study (Macias et al.) that examined early mortality showed that early death due to sepsis was more likely to result from refractory shock.[125] They also found early death to be associated with severe deficiency (<40%) in protein C levels (a physiological anticoagulant) and increased interleukin 6, a molecule involved in mediating the immune system.[125] This information may not be relevant for patients with

IPD, as this study focused on sepsis and only a small proportion of cases would have been caused by *S. pneumoniae.*

The paper by Marrie et al. was published in 2011, after the initial search was performed. This paper examined IPD in Alberta adults from 2000 to 2004 and included data from the CASPER study. The study by Marrie et al. included analysis of potential risk factors for overall mortality as well as risk factors for early mortality. The Alberta IPD study utilized a case report form based on the original CASPER case report form and coordination of the chart reviews was conducted by CASPER staff. However, for analysis and publication, all data from the Alberta study was analyzed by Dr. Marrie and his research staff and the CASPER component of the Alberta data was merely provided to Dr. Marrie in raw form. One CASPER investigator (Dr. Otto Vanderkooi) was an author on the Marrie paper.

Marrie et al. found that age 18-40 years was protective against both 30-day and 5-day mortality.[126] Mechanical ventilation, altered mental status, and cardiac arrest were risk factors for both 30- and 5-day mortality.[126] High mortality serotypes were also predictive of mortality.[126] However, it appears the serotypes were categorized based on the mortality caused in this population, which would, in a circular manner, lead to an association with mortality.

The current study had a more complete analysis for several reasons. First of all, Marrie et al. did a separate analysis for mortality at 30 days and mortality at 5 days, rather than including all outcomes (survival, 5-day mortality, and 30-day mortality) in the same analysis. The use of separate analyses causes loss of information. It is unclear whether the 5-day mortality was grouped in with the 30-day mortality in the study by Marrie et al. If they were grouped, the 5-day mortality may be driving the risk factors found in the overall mortality. Second, Marrie et al. chose to include in the multivariable analysis all factors that had a p-value of <0.25 in the univariable analysis. It is better to decide *a priori* which factors will be included in a model, as the results may be very different in a univariable analysis than in an adjusted analysis with regards to what is

significant. Third, Marrie et al. adjusted for individual underlying illnesses, which would be preferred ideally, but with the sample size available they could not have had enough people to effectively adjust for so many different covariates in their multivariable model. Fourth, Marrie et al. grouped age rather than keeping it as a continuous variable in the multivariable model, which can result in loss of information. Finally, it appears that Marrie et al. did not consider interactions (such as effect modification) in their model, they only accounted for possible confounding.

2.8 Study Purpose

Although *S. pneumoniae* is a well-studied pathogen, there is a need for research examining the combined influences of clinical, microbiological, and patient-related factors on IPD-related mortality. To our knowledge, no current studies compare how the interactions between these variables may differ between early mortality, late mortality, and survival in a well-adjusted, multinomial logistic regression analysis. The current study investigated *S. pneumoniae* disease specifically and included all forms of IPD. There may be other interventions to consider to complement antibiotic and supportive therapy in decreasing the proportion of deaths that occur in the first 5 days, and therefore decrease the overall case-fatality rate. However, in order to determine this we must first understand how and when these factors come into play. The purpose of this study was to better understand factors involved in early and late mortality in patients with IPD.

Chapter Three: Methods

3.1 Data collection

The Calgary Area *Streptococcus pneumoniae* Epidemiology Research (CASPER) network is an ongoing prospective population-based surveillance network that collects data on all patients with IPD detected through active surveillance by the Calgary Laboratory Services (CLS). CLS serves the entire Calgary and area Zone of Alberta Health Services; therefore, all IPD cases presenting to a Calgary hospital or health center are captured. IPD is defined as acute illness with a positive culture of *S. pneumoniae* isolated from a normally sterile site (e.g. blood, cerebrospinal fluid, pleural fluid).

When a positive diagnosis is confirmed by CLS, the CASPER team is notified and promptly contacts the patient to inquire about their interest in participating in the study. If the patient consents, a chart review and in-person interview are conducted. The interview and chart review are completed using a standardized questionnaire and case report form respectively. If a patient dies before an interview can take place, a chart review is done and an autopsy report is requested from the medical examiner. If possible, an interview with the patient's next of kin is also carried out. If a patient or their next of kin refuses consent, neither a chart review nor interview is performed and only basic demographic information is recorded from the laboratory forms and from the Alberta Health and Wellness Notifiable Disease Report Form (NDR) which is completed by a designated staff at each hospital, given that IPD is considered a notifiable disease in Alberta. If a patient cannot be reached for consent after three attempts via phone and/or email, only a chart review is completed.

For the current study, if more than one *S. pneumoniae* isolate was obtained from one patient during a single episode, only one isolate was included. If a non-blood sterile site isolate was identified in addition to blood (e.g. CSF), the non-blood isolate was included rather than the blood isolate. Blood invasion will occur prior to the less likely invasion of another site such as the meninges or pleural space. Typically, the same bacterial strains that are isolated from the blood will be isolated from the non-blood site. Therefore, the

non-blood isolate was chosen over the blood isolate because these samples reflect more focal and specific disease than bacteremia alone and invasion of these sites is preceded by invasion of the blood. If a patient had more than one episode of IPD more than 30 days apart, they were considered to be separate episodes.

3.1.1 Laboratory Isolation

When blood cultures are collected, they are placed into 2 bottles (1 aerobic, 1 anaerobic) containing broth, sodium polyanetholesulfonate (SPS) anticoagulant, and resin. The purpose of the resin is to attach to growth inhibitors such as antibiotics. The bottles are sent to the CLS laboratory where they are placed on a Bac-T-Alert (bioMérieux, France) system for incubation at 35ºC in ambient atmosphere. Growth is indicated by an increase in gas pressure and a colour change in the indicator in the bottom of the bottle. The Bac-T-Alert flags the bottle as having growth and the bottle is removed and a sample is set up for a Gram-stain then plated automatically on Blood Agar, Chocolate Agar, MacConkey Agar, CNA Blood Agar and Brucella Agar, then incubated at 35ºC in the appropriate atmosphere for the medium (see appendix B) for 4 days before calling no growth. Extra media may be plated according to what is seen on the Gram stain.

With sterile fluid cultures (e.g. pleural fluid) if there is ≥ 5 mL of fluid, some of the fluid is planted to blood culture bottles and placed on the Bac-T-Alert. However, CSF is never planted to bottles. Fluid cultures are cytospun for Gram stain, then planted automatically onto Blood Agar, Chocolate Agar, and MacConkey Agar and incubated at 35ºC in appropriate atmosphere for the agar (see appendix B). These are incubated 4 days before calling no growth. Other types of media may be included if required or requested.

3.1.2 Laboratory Identification

CLS staff alert CASPER to all clinical isolates positive for *S. pneumoniae*. Following plating of the sample, CLS tests for the presence of *S. pneumoniae* using colony morphology, alpha haemolysis, optochin susceptibility and bile solubility. When *S. pneumoniae* is plated and incubated on blood agar, there is growth of colonies of variable appearance (smooth, rough or mucoid), and there is lysis of blood cells in the agar resulting in a green zone of clearance (alpha haemolysis). *Streptococcus pneumoniae* is sensitive to optochin (ethylhydrocupreine hydrochloride) about 95% of the time, while other alpha-hemolytic streptococci are usually resistant.[31] A sensitive screen is indicated by a zone of no bacterial growth (zone of inhibition) ≥14mm around the optochin disk.[31] If the optochin screen is sensitive then the isolate must be positive for one of Phadebact PneumoSlide Kit (Sparks, MD) or bile solubility for the lab to label it as *S. pneumoniae*. *Streptococcus pneumoniae* is susceptible to lysis by bile salts, while other alpha-haemolytic streptococcus are resistant. If the Optochin screen is intermediate resistance (7-6 mm zone), then the isolate must be positive for both the PneumoSlide and bile solubility for CLS to label the sample *S. pneumoniae*. If Optochin is Resistant (6 mm or no zone of inhibition), then CLS will perform both the PneumoSlide and bile solubility tests, but the microbiologist on call must be consulted prior to identifying the isolate as *S. pneumoniae*.

3.1.3 Antibiotic Susceptibility

Antibiotic susceptibility profiles are determined by broth micro-dilution using the Clinical and Laboratory Standards Institute (CLSI) guidelines.[128] An inoculum in 0.85% saline is prepared according to MacFarland standard. Then 100uL of the inoculum is added to cation adjusted Mueller Hinton broth with lysed horse blood (2.5-5% v/v), which is mixed and added to an inoculation tray. A RENOK Rehydrating Inoculator is used to add the appropriate volume of inoculated broth to the MicroScan Strep Plus 1

panels (Siemens Healthcare Diagnostics, Illinois). A known concentration of antibiotic is present in the MicroScan plates (predetermined by the manufacturer), and the laboratory adds a known concentration of *S. pneumoniae* cells. The MicroScan plates are then incubated at 35 ± 2 ºC in ambient air for 20-24 hours. The lowest concentration of antibiotics on the MicroScan panel that inhibits the bacterial growth is taken as the minimum inhibitory concentration (MIC).

3.1.4 Serotyping Methods

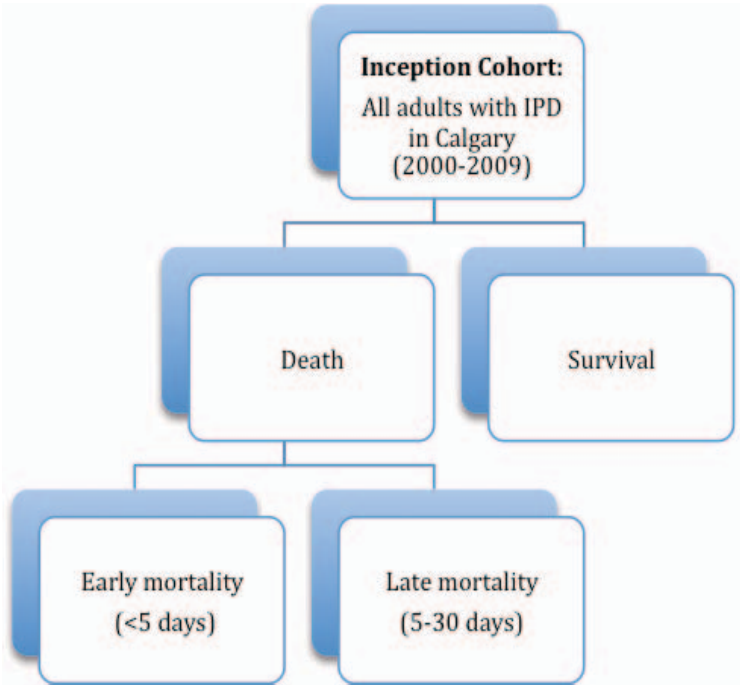
S. pneumoniae serotype was determined on the basis of a positive Quellung reaction using serotype-specific commercial antisera from Statens Serum Institute (Copenhagen, Denmark).[129] Serotyping was performed as part of routine testing on all IPD isolates at the Provincial Laboratory for Public Health in Edmonton, Alberta (formerly designated as the National Centre for Streptococcus until 2010).

3.2 Sampling

All adult cases (18 years of age and older) of community-acquired and hospital-acquired IPD identified from January 1, 2000 to December 31, 2009 were included. If the invasive infection was isolated from a culture obtained 48 hours or more after hospital admission, then it was considered hospital-acquired and the culture date and time were used as the episode start date and time. Otherwise, the date and time of presentation to a health facility were used as the episode start. Subjects living outside the Calgary zone were excluded. The Calgary zone includes the geographic area previously known as the Calgary Health Region, which encompasses the population living in Calgary and the surrounding towns that are served by Calgary hospitals. It should be noted that the 405 patients from CASPER data who presented between 2000 and 2004 were included as part of the Alberta-wide sample used in the study by Marrie et al.[126]

The current study used a population-based prospective cohort design using CASPER data. Three groups were defined: early mortality, late mortality, and survivors. The early mortality group consisted of patients with IPD in whom mortality occurred fewer than 5 days after presentation with the invasive infection. Late mortality was defined as those in whom mortality occurred 5 to 30 days after presentation with the invasive infection. The third group consisted of patients who survived the IPD infection for 30 days, which is the limit of time for which data on cases was collected. These outcomes were considered to be nominal.

Figure 2. Sampling method from CASPER data



3.3 Definitions

3.3.1 Definition of All-Cause Mortality

All participants with IPD who died within 30 days of presentation to hospital were included in the mortality group. Those who were alive 30 days after presentation were considered to be survivors. It may be difficult to distinguish whether death within 30 days was due to IPD or another cause, as an infection can weaken the body's ability to cope with other illnesses. Therefore, the definition for mortality included anyone who died within 30 days of presentation regardless of whether the cause was considered to be the infection or not.

3.3.2 Definition of Early Mortality

In Austrian and Gold's study in 1952-1962, 5 days was the point at which there was a natural decline in the proportion of deaths occurring. In a more recent study in 2008, Garcia-Vidal et al. found a difference in risk factors associated with early mortality due to all-cause CAP when they used a cut-off of 2 days for early mortality.[123] When Garcia-Vidal et al. looked at 5 days as the cut off for early mortality they did not see a difference in risk factors between early and late mortality.[123] For this study, we investigated where the most pronounced decline in survival was in our data by generating a Kaplan-Meier survival curve. The most pronounced decline observed in the data was used as the final definition of early mortality for this study. This definition was set prior to any descriptive or multivariable analysis on the data.

This analysis to define early mortality gives our results greater clinical relevance. The purpose of the current study was not to find a difference between early and late mortality. The purpose was to define early mortality clinically by visualizing the natural breakpoint in the data, and to then examine factors that influence early and late mortality. We then hoped to find factors that could be focused on clinically to reduce mortality.

The crude Kaplan-Meier curve (Figure 3) showed a high proportion of deaths between days 0 and 4. This fits with our predicted definition for early mortality, which was set as less than five days after presentation.

A histogram of the distribution of deaths from time of presentation to death showed a similar cut off point with the greatest density of mortality occurring in the first 4 days (Figure 4).

Figure 3: Kaplan-Meir Survival Curve Indicating Days to Mortality or Censorship at 30 Days (N=1001)

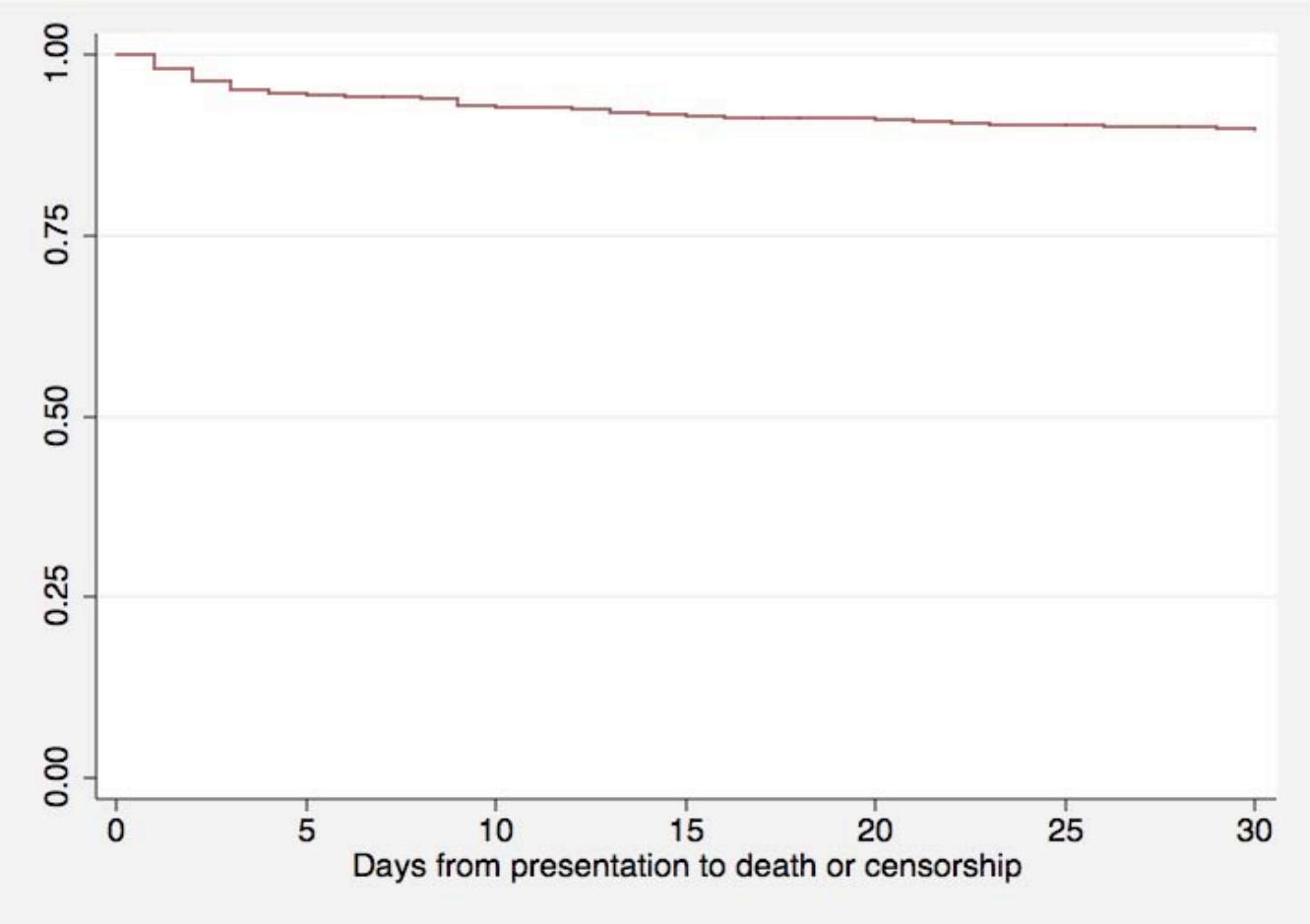
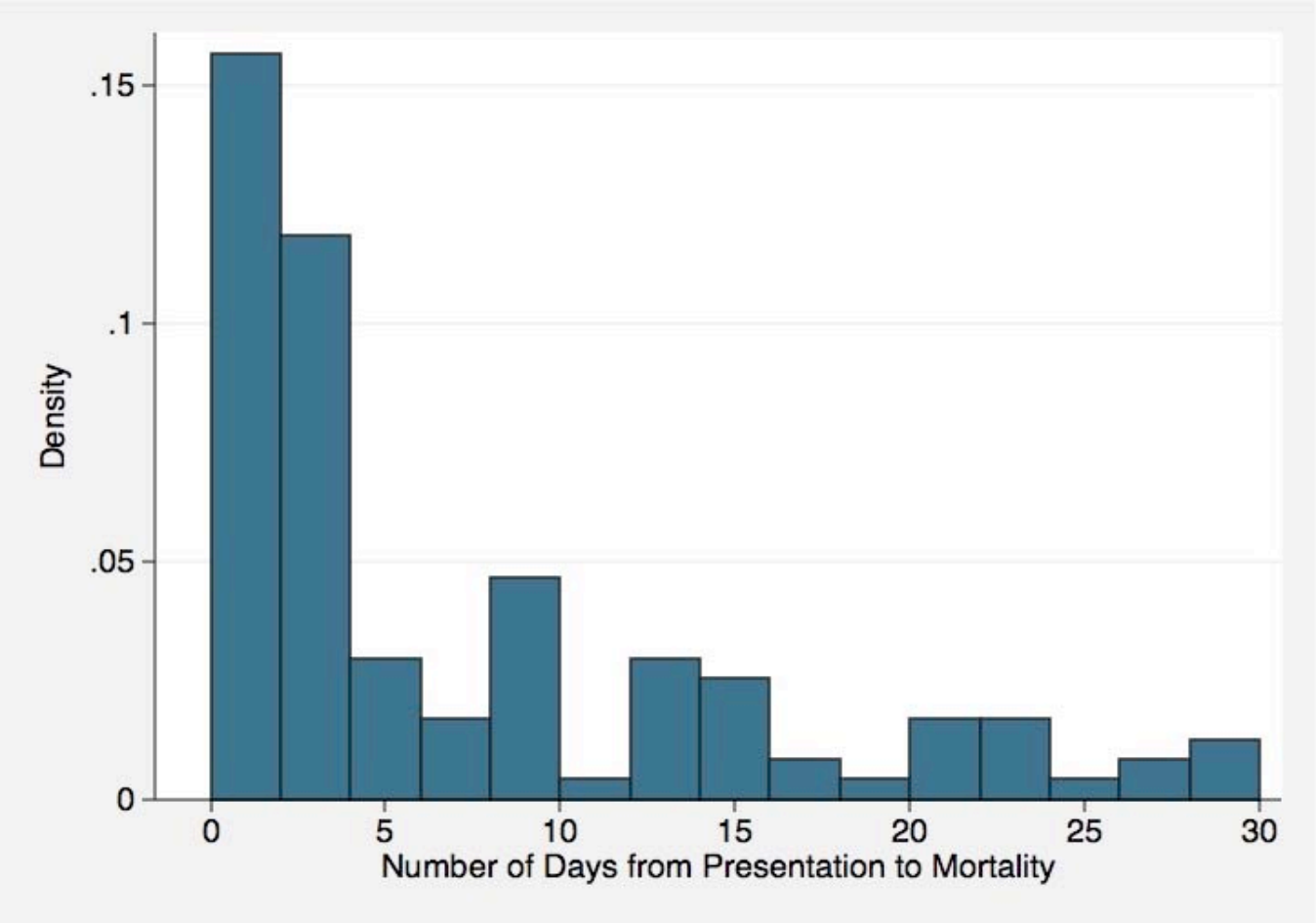


Figure 4: Histogram Showing Number of Days from Presentation to Death Among Patients Who Died (Box width=2 days, N=118)



3.3.3 Definition of Severity

Patients admitted to an intensive care unit (ICU) or mechanically ventilated during hospital admission were considered to have severe disease. These measures were proxies for the disease severity, and used as exposure (the predictive variable) in the regression model.

3.4 Epidemiological Definitions

3.4.1 Biases

Due to the uncontrolled, observational nature of epidemiological research, it is prone to bias. Selection bias and misclassification bias are particularly important in data collection and cannot be corrected for in the analysis, whereas confounding bias can be adjusted for in the analysis provided the confounder is known and measured.[130]

3.4.1.1 Selection Bias

Selection bias is a distortion of the true effect estimate (e.g. risk ratio) due to how participants are selected into a study or differential participation in the study.[130] If selection into the study varies between groups in a way that is associated with both outcome and exposure, it can result in an effect estimate that is different in the study population than in the true population.

3.4.1.2 Misclassification Bias

Misclassification or information bias can occur when subjects are misclassified with regards to either exposure or outcome.[130] Non-differential misclassification bias occurs when the misclassification is not associated with the outcome and exposure (i.e. both levels of exposure and outcome are misclassified), resulting in dilution of the effect

estimate towards the null.[130] Differential misclassification occurs when the misclassification is associated with both exposure and outcome (only one level of exposure/outcome is misclassified), which results in a biased effect estimate that may be more or less extreme than the true effect.[130] At times the patients who experience an outcome may have more (or less) information available, resulting in differences in completeness of the information collected, which may result in bias. If missing information is equivalent for both outcomes and does not vary across exposure groups then the effect estimate may be unbiased.

3.4.1.3 Confounding Bias

Confounding bias occurs when an unmeasured factor is causing a distorted relationship between outcome and exposure.[130] A confounder is a factor that is associated with both the outcome and exposure but is not on the causal pathway and it may distort the apparent effect estimate from the true relationship between outcome and exposure.[130] This bias can be dealt with by measuring the external factor and adjusting for its effects in the analysis. Adjusted analysis can be done by stratifying the exposure-outcome relationship by the different levels of the confounder (stratified analysis), or by using multivariable analysis where the effects of multiple factors can be accounted for simultaneously.

3.4.2 Effect Measure Modification

Certain clinical, microbiological and patient-related factors may influence the relationship between an outcome and a predictive variable (the exposure). As a result these factors are included as covariates in the multivariable model to account for potential confounding bias or effect measure modification.

Variables that act as effect measure modifiers change the relationship between the exposure and outcome so that the risk estimate (e.g. risk ratio) due to exposure is different for different levels of the measured variable (the effect modifier). For example,

if gender modifies the effect of disease severity on death, then severity in males will have a different risk estimate than severity in females. Severity may be associated with increased risk of death in males, while in females severity is not associated with risk of death at all. If this were the case, the crude risk estimate may be significant, but in reality severity is only a risk factor for males, and this will only be seen if the estimate is stratified by gender.

Interaction terms are included in a multivariable model to account for potential effect modification. Pair-wise interaction terms in a statistical model are represented by the multiplication of the exposure (e.g. severity) by the measured variable (e.g. gender). This creates a new term that allows for each level of severity by each level of gender. If the interaction term is significant, then severity and gender can no longer be considered alone, they must be considered together as interacting variables, and the reported measure of effect (e.g. risk ratio), will be reported separately for each gender and severity level within the multivariable model. That is, if severity is dichotomized into severe disease and less severe disease, and females with less severe disease are considered to be baseline, then a separate risk ratio will be reported for females with severe disease, males with severe disease, and males with less severe disease.

It is important to include covariates and interaction terms in the analysis to ensure that confounding or effect modification are not causing or distorting the results. The following factors were discussed *a priori* as covariates to potentially include in the multinomial regression model based on clinical relevance and past literature.

3.5 Covariate Selection

3.5.1 Age

Age is a necessary variable to adjust for because it can act as a confounder of many disease-outcome relationships. Elderly people are at greater risk of death, and may also have higher chance of being admitted to ICU, which is a proxy for severity in this study. Therefore, age was adjusted for as a continuous variable in the multinomial regression

model and as ordinal age groups of 18-64, 65-84, and 85+ years for the stratified analysis. By using age as a continuous variable in the multinomial model, there is no loss of information; however, it does assume a linear relationship between age and mortality. For descriptive analyses the mean age was considered.

3.5.2 Gender

There is some evidence that men have a higher case fatality rate due to IPD than women.[12, 103] The data on gender for this study is complete and it is a common confounder of many different diseases; therefore, it was included in the multinomial regression.

3.5.3 Comorbidities

The presence of a comorbidity in a patient can increase the likelihood of death and ICU admission. Therefore, it is necessary to adjust for comorbidities. In order to be more efficient in the analysis of comorbidities, a modified Charlson comorbidity index was developed by matching the comprehensive list of comorbidities collected in the CASPER database to the classification methods used by Charlson et al.[131] A Charlson comorbidity index gives greater weight to more immunocompromising conditions such as solid malignancies, haematological cancers, and HIV. This is appropriate for IPD, as evidence suggests that solid organ malignancies, haematological cancers, and HIV significantly increase risk of death among patients with IPD.[92] Appendix C.1 shows how the CASPER measured comorbidities were grouped into Charlson comorbidity classifications. Classification of cancers from the CASPER database into the Charlson cancer classifications is shown in Appendix C.2.

The survival curve shown in the paper by Charlson et al. suggests that the greatest change in risk of mortality occurs between a Charlson of 0 and 1.[131] Therefore, for the sake of descriptive analyses, the proportion of deaths among people with a Charlson of 0

was compared to those with a Charlson of ≥1. For multinomial regression the Charlson index was divided into three groups with a comorbidity index of 0, 1, or ≥2. This was a clinically relevant breakdown for the data and allowed less loss of information than a dichotomous variable.

3.5.4 Smoking Status

There is evidence that smoking is an independent risk factor for developing IPD; however, there is no clear evidence of increased mortality risk in smokers with IPD.[93] Smoking is commonly associated with morbidity and mortality; therefore, it was included in the analysis despite 11% of patients missing this variable in the data. For analysis, smoking status was divided into four nominal groups: current smoker, former smoker, never smoker, and unknown.

3.5.5 Alcoholism

In the CASPER dataset alcoholism is based on self-report in the interview and any information found in the chart review. Alcoholism is not consistently recorded in patient charts; therefore, this variable may have been prone to misclassification bias. Alcoholism has been shown to be a risk factor for IPD; however, it has not been associated with increased risk of mortality.[14, 89, 113] Therefore, it would be unlikely to confound our results. Alcoholism was not included in the analyses for this study.

3.5.6 Disease Manifestation (Primary Diagnosis)

Meningitis has a higher mortality rate than pneumonia or bacteraemia and is also likely to be associated with increased disease severity; therefore, it is important to adjust for primary diagnosis.

Patients may have received a clinical diagnosis of more than one disease manifestation, such as pneumonia and bacteraemia, or meningitis and bacteraemia. We followed a hierarchy with the highest severity diagnosis first: meningitis, empyema, pneumonia, other invasive, and bacteraemia. If a patient had multiple diagnoses, the highest in the hierarchy was assigned as the primary diagnosis.

The primary diagnosis used for data analysis was based on both laboratory results and clinical diagnosis. In cases where laboratory data did not clearly support the clinical diagnosis of meningitis (i.e. no sampling of CSF or negative culture from CSF) other laboratory features were examined more closely to ensure correct classification of meningitis based on the minimum criteria defined by the World Health Organization:

1. A positive CSF culture, regardless of CSF parameters or
2. A positive blood culture and 5 or more WBC in the CSF or
3. A positive blood culture and decreased glucose in CSF, or increased protein in CSF.[132]

The "other invasive" classification included patients with cultures from sites such as joint fluid and peritoneal fluid. The six samples that were labelled "other invasive" were checked to ensure that they were true invasive samples. All of them were invasive and included aqueous eye aspirate, ascitic fluid, and fine needle aspiration from a lymph node.

We classified patients as invasive disease only if the culture samples were taken with sterile technique to ensure results were not due to contamination during sample collection.

For multinomial regression the primary diagnoses were divided into 3 categories: meningitis, pneumonia/empyema, or bacteraemia/other invasive, with bacteremia/other invasive as baseline.

3.5.7 Treatment and Antibiotic Susceptibility

Antibacterial treatment encompasses several important considerations. The type of antibiotic therapy given is important, but so are the dose, route of administration, antibiotic susceptibility of the *S. pneumoniae* strain, and time from presentation to receipt of antibiotic.

In an attempt to capture all of these factors, time from presentation with IPD to receipt of first appropriate treatment measured in hours was used. The time to appropriate treatment was then categorized into 4 groups: appropriate treatment within <24 hours, 24-48 hours, >48 hours, or no appropriate treatment received. Receipt of appropriate antibiotic treatment within 48 hours has been suggested to decrease mortality in patients with CAP.[133]

3.5.7.1 Definition of Appropriate Antibiotic Treatment

Appropriate treatment was defined as receiving an antibiotic appropriate for the *S. pneumoniae* strain causing infection. This included the general appropriateness of the antibiotic and route of administration for treatment of *S. pneumoniae* (table 2), as well as the susceptibility of that particular strain. If the infecting strain was not susceptible to an antibiotic then that antibiotic was considered not appropriate for that particular patient. Antibiotic susceptibility cut-offs were based on the current CLSI MIC cut offs (table 3).[55] This included considering the different MICs for those patients with meningitis compared to other IPD manifestations. Higher doses of antibiotics are needed to ensure that the required MIC is reached in the CSF due to the lower penetration of antibiotics across the blood-brain barrier.

3.5.7.2 Creating Antibiotic Appropriateness Variable

In 2005, the Calgary Laboratory Services switched MIC panels used for testing *S. pneumoniae* susceptibilities. The CLS performed both new and old MIC panels on 25 patients across 2005/2006. It was determined *a priori* that the two different panel results should have been within one doubling dilution of one another, in which case they could be considered equivalent. For most of the MIC results for the 25 patients with both panels, the dilutions were equal or within one doubling dilution. Those that were more than one doubling dilution off were checked to ensure it was not a data entry error; however, the panels were not re-done. The higher dilution was used for all panel results where the MICs were not identical. From a clinical perspective, it is a greater error to label a bacterial strain as susceptible if it is actually resistant.

For creation of the time to appropriate treatment variable, we classified all strains with intermediate or resistant MICs as non-susceptible. If a strain was non-susceptible to an antibiotic, then that antibiotic was considered not appropriate as a treatment and was classified accordingly. However, the dose of antibiotic was not considered.

If the patient had meningitis and the lab did not test MICs for a particular antibiotic then the antibiotic was considered inappropriate. Susceptibility of certain antibiotics can be inferred from other antibiotic MICs, but only for non-meningitis cases. The following MICs and inference rules were applied for this variable as per CLSI guidelines and are outlined in table 3.[55] Erythromycin MICs were used to predict sensitivity to azithromycin and clarithromycin, as per the CLSI guidelines.[55] If *S. pneumoniae* is sensitive to penicillin and the disease manifestation was not meningitis then that strain can be assumed susceptible to certain other beta-lactam antibiotics. However, if the strain is not penicillin sensitive, then it is necessary to do further testing for sensitivity to other beta-lactams. A number of patients received piperacillin-tazobactam, which CLS did not test MICs for. Therefore, as piperacillin-tazobactam can be inferred from ampicillin, the same penicillin cut-offs were used for piperacillin-tazobactam as for ampicillin. If the

penicillin MIC was 0.06μg/L or less, then susceptibility to piperacillin-tazobactam was assumed.[55]

Cloxacillin, cefazolin, and ceftazidime are not considered to be first choice antibiotics for *S. pneumoniae,* although they may be potentially effective. For non-meningitis cases, a penicillin MIC of ≤ 0.06μg/L was considered to infer susceptibility to cloxacillin, cefazolin and ceftazidime according to the input of two infectious disease physicians (Drs. Jim Kellner and Otto Vanderkooi). If the MIC was >0.06μg/L or the patient had meningitis, then these antibiotics were considered to be inappropriate.

Antibiotics that were classified as appropriate for treatment of meningitis or non-meningitis manifestations of IPD are shown in table 2. Due to the rapidly changing recommendations and differences in susceptibility patterns in different geographic areas, these classifications were based on the clinical knowledge and experience of 3 physicians (Drs. Jim Kellner, Otto Vanderkooi and Kevin Laupland), as well as on literature and current recommendations at the time this research project was underway.

Figure 5. Classification of Antibiotic Appropriateness

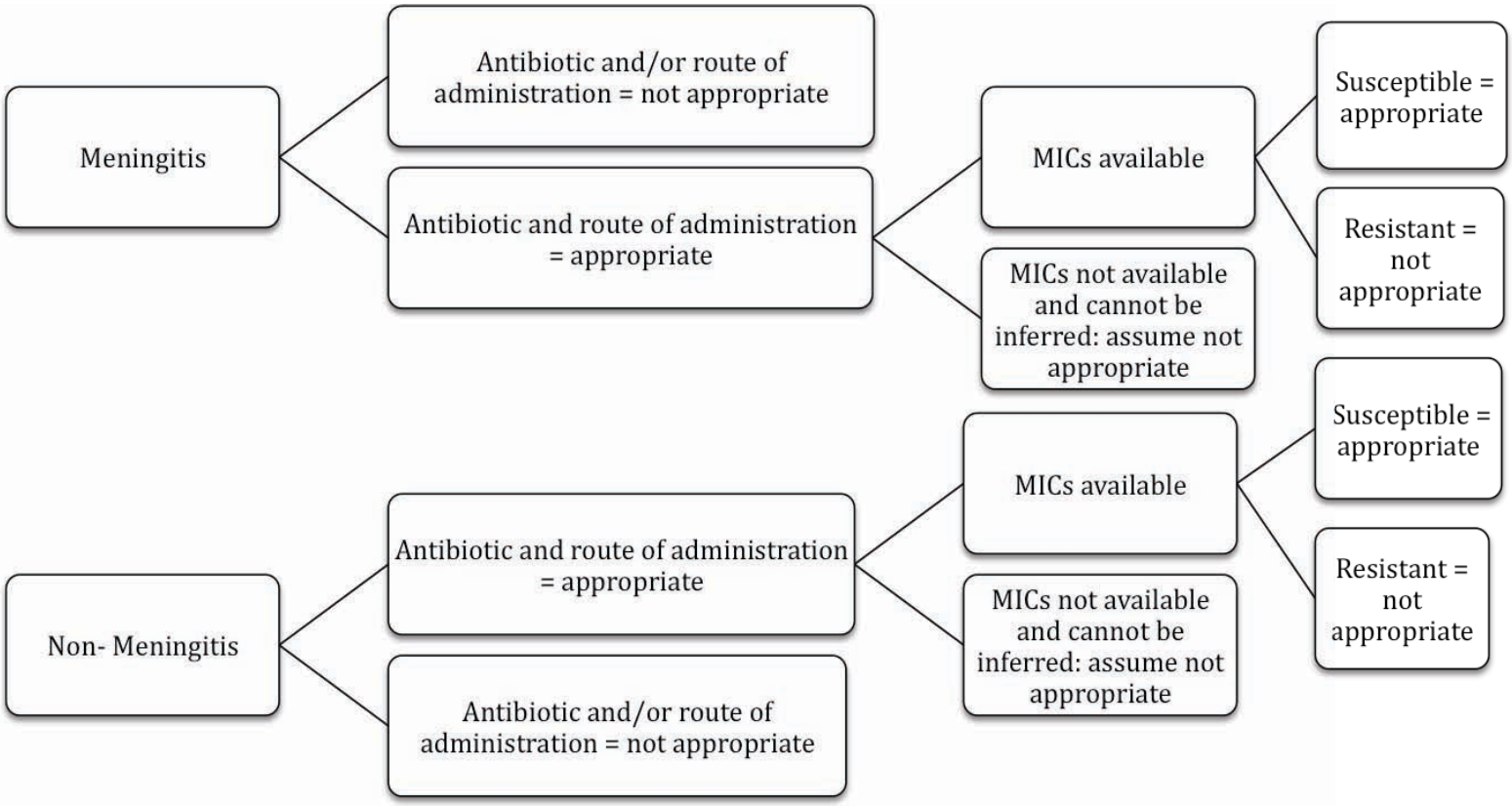


Table 2. Antibiotics Considered Appropriate for Treatment of *S. pneumoniae* Meningitis and Non-Meningitis

Route of Administration	Meningitis	Non-Meningitis
Parenteral	Ampicillin	Ampicillin
	Cefotaxime	Azithromycin*
	Ceftazidime	Cefazolin
	Ceftriaxone	Cefotaxime
	Clindamycin*	Ceftazidime
	Cloxacillin	Ceftriaxone
	Gatifloxacin	Cefuroxime
	Levofloxacin	Clarithromycin*
	Meropenem	Clindamycin
	Penicillin	Cloxacillin
	Vancomycin	Gatifloxicin

Route of Administration	Meningitis	Non-Meningitis
		Levofloxacin
		Meropenem
		Penicillin
		Piperacillin
		Piperacillin-tazobactam (Tazocin)
		Trimethoprim-sulfamethoxazole (TMP/SMX)
		Vancomycin
		Linezolid
		Erytromycin*
Oral		Azithromycin*
		Clindamycin*

Route of Administration	Meningitis	Non-Meningitis
		Gatifloxicin
		Levofloxacin
		Erythromycin*
		Linezolid

*= Only appropriate as second drug in dual therapy

Table 3. Antibiotic Susceptibility MIC Cut-Offs (CLSI 2011)[55]

Antibiotic	MIC Meningitis			MIC Non-Meningitis		
	S	**I**	**R**	**S**	**I**	**R**
Penicillin (parenteral)	≤ 0.06 μg/L	-	≥ 0.12 μg/L	≤ 2.0 μg/L	4.0 μg/L	≥ 8.0 μg/L
Ampicillin	Not tested, could not be inferred	Not tested, could not be inferred	Not tested, could not be inferred	Penicillin MIC ≤ 0.06 μg/L	Not tested, could not be inferred	Not tested, could not be inferred
Cefotaxime	≤ 0.5 μg/L	1.0 μg/L	≥ 2.0 μg/L	≤ 1.0 μg/L	2.0 μg/L	≥ 4.0 μg/L
Ceftriaxone	≤ 0.5 μg/L	1.0 μg/L	≥ 2.0 μg/L	≤ 1.0 μg/L	2.0 μg/L	≥ 4.0 μg/L
Cefuroxime	Not appropriate	Not appropriate	Not appropriate	≤ 0.5 μg/L	1.0 μg/L	≥ 2.0 μg/L
Meropenem	≤ 0.25 μg/L	0.5 μg/L	≥ 1.0 μg/L	≤ 0.25 μg/L	0.5 μg/L	≥ 1.0 μg/L

Antibiotic	MIC Meningitis			MIC Non-Meningitis		
	S	**I**	**R**	**S**	**I**	**R**
Vancomycin	≤ 1.0 µg/L	-	-	≤ 1.0 µg/L	-	-
Erythromycin	≤ 0.25 µg/L	0.5 µg/L	≥ 1.0 µg/L	≤ 0.25 µg/L	0.5 µg/L	≥ 1.0 µg/L
Azithromycin	Erythromycin MIC ≤ 0.25 µg/L	Erythromycin MIC 0.5 µg/L	Erythromycin MIC ≥ 1.0 µg/L	Erythromycin MIC ≤ 0.25 µg/L	Erythromycin MIC 0.5 µg/L	Erythromycin MIC ≥ 1.0 µg/L
Clarithromycin	Erythromycin MIC ≤ 0.25 µg/L	Erythromycin MIC 0.5 µg/L	Erythromycin MIC ≥ 1.0 µg/L	Erythromycin MIC ≤ 0.25 µg/L	Erythromycin MIC 0.5 µg/L	Erythromycin MIC ≥ 1.0 µg/L
Levofloxacin	≤ 2.0 µg/L	4.0 µg/L	≥ 8.0 µg/L	≤ 2.0 µg/L	4.0 µg/L	≥ 8.0 µg/L
Gatifloxacin	≤ 1.0 µg/L	2.0 µg/L	≥ 4.0 µg/L	≤ 1.0 µg/L	2.0 µg/L	≥ 4.0 µg/L

3.5.8 Serotype

Serotype was not included in the multinomial analysis for the current research. Individual serotypes have been found to be associated with mortality relative to other individual serotypes.[12, 18] However, the data for this study included only a small, or relatively small, number of each individual serotype, which made it impossible to consider serotypes separately with regards to mortality. Furthermore, it is to be expected that if a serotype with a high propensity for causing death is compared to a serotype with a low propensity for causing death, an association will be apparent. For instance, Harboe et al. appear to have conducted several binomial logistic regressions comparing each serotype separately to a reference serotype (serotype 1), which has a low association with death.[12] Similarly, Weinberger et al. analysed serotypes relative to serotype 14 to show an association with risk of death.[18] Martens et al. considered only the serotype most associated with death (serotype 3) and least associated with death (serotype 1) according to their univariable analysis, and analysed them in a multivariable regression with "Other" serotypes as the reference group.[14] Naturally, when analysing this way, serotype 1 appeared as protective against death and serotype 3 was associated with increased risk of death.[14] Inevitably, when analysing one serotype with a high tendency to cause death against a serotype with a low tendency to cause death, there will be a significant difference between the two. Alternatively, when serotypes are pooled and analysed along with host factors this may not be the case. Invasive pneumococcal disease is caused by a diversity of serotypes and it is unlikely that single serotypes would have a large confounding effect on the current study results as a whole if not adjusted for.

Harboe et al. showed a strong association between host risk factors and death as well as serotype, making it clear that although some serotypes are more associated with death than others, host risk factors have an important role.[12] Knowledge of the infecting serotype does not change the clinical management of IPD, while host factors such as comorbidities may be controlled and treated to increase a patient's chance of survival. When serotypes were analysed as a group, Alanee et al. found host factors to be more

associated with death than serotype groups.[21] Furthermore, there is no effective way to group serotypes for adults in order to appropriately adjust for them in a model. Grouping by PCV7 vaccine serotype is not ideal, because PCV7 vaccine serotypes are prevalent in children, not adults. Therefore it does not make sense to group by PCV7 vaccine serotype in a study that focuses on adults. PPV-23 includes all the serotypes most common in adults, therefore grouping this way would have resulted in most serotypes in one group, which would have told us very little. Changes in serotype prevalence due to the introduction of new vaccines could affect this variable.

There has been some research to suggest that serotypes may be associated with certain clinical presentations; however, the model was adjusted for primary diagnosis, therefore this should be sufficient when the outcome is death. If the outcome were disease manifestation, it may be more important to consider serotype.

A univariable, descriptive analysis of serotype distributions was done, but serotype was not included in the multivariable analysis *a priori*.

3.6 Data Analysis

Data analysis for the current study involved descriptive analysis with tests of proportions as well as calculations of incidence rates and serotype proportions. Multivariable analysis was conducted using multinomial regression with three outcomes: survival, late mortality (5 to 30 days after initial infection) and early mortality (fewer than 5 days after initial infection).

3.6.1 Descriptive Analysis

Incidence rates per year over the study period of 2000-2009 were generated. Proportions of mortality to survival, and early mortality to late mortality were compared using tests of proportions or t-tests for all variables that were chosen to be included in the multinomial analyses (age, gender, smoking status, Charlson index, time to appropriate antibiotics,

and primary diagnosis). The distribution of serotypes in the population was described using proportions and tests of proportions to compare PCV7 to non-PCV7 serotype frequencies. Hypothesis tests of difference in proportions were used and 95% CI and p-values are presented. However, the results of these comparisons can be only be considered independently, that is, with respect to the variable at hand, ignoring all other variables, and are useful only for describing the data. Only limited inferences can be made from descriptive analyses.

For the continuous variable, means were compared using t-tests after determining that the mean best represented the age distribution of the population.

3.6.2 Stratified Analysis

Stratified analyses were performed for all variables that were chosen for use in the multinomial model. Separate stratified analyses were performed to compare survivors to all cause mortality, and early mortality to late mortality. A significance level (alpha) of 0.05 was used in all tests.

Stratified analyses were performed to look at how age (categorized), Charlson comorbidity index, smoking status, gender, primary diagnosis, and time to appropriate antibiotic treatment may modify or confound the relationship between disease severity and risk of mortality.

3.6.3 Multivariable Analysis

The data was analyzed using a multinomial logistic regression model with 3 outcomes: survival, late mortality, and early mortality. A multinomial regression model allows analysis of a dependent variable that is categorical and has more than two levels. In order to do this, a baseline outcome level is selected and then the model constructs comparisons between the baseline level and each of the other outcome categories. Because the model

contains multiple levels of outcomes, the resulting coefficients are ratios of risk ratios, or relative risk ratios.

3.6.3.1 Predictor Variables

ICU admission and mechanical ventilation were used as proxies for disease severity, which was the main predictor variable in the multinomial logistic regression. All factors that may influence the outcome (e.g. age, gender, disease severity) are predictor variables, with the exposure of interest being the main predictor variable. The purpose of this study was to look at multiple potentially independent factors that may influence mortality. However, disease severity was chosen to be the main predictor variable in the model and the other factors were considered as potential confounders, independent risk factors, and effect modifiers. This meant that in the stratified analysis the relationship between the predictor variable (severity) and mortality was considered for different levels of the other factors (e.g. age categories) to look for effect modification.

In the multivariable analysis, all potential risk factors are entered in the model simultaneously (the main predictor variable and the other factors), so that if any of the other factors are independent risk factors, this will be apparent. However, the main predictor variable is also entered in the model combined with each of the other factors in interaction terms to look for effect modification by the other factors on the relationship between the predictor variable and the outcome. Interaction is explained further in section 3.6.3.3. Multivariable regression is used to examine the relationship of predictor variables with the observed outcome (the dependent variable).

3.6.3.2 Covariates

The multinomial model included the main predictor variable (severity) as well as 6 other predictor variables that may act as confounders, effect modifiers or independent risk factors: age, gender, comorbidities (using Charlson index), smoking status, primary

diagnosis, and time to appropriate antibiotic treatment. These other predictor variables are considered to be the covariates because they are typically considered to be secondary to the main predictor variable. The common rule of needing 10 outcome occurrences in the smallest outcome group per variable included in the model was relaxed to 5 based on the paper by Vittinghoff et al.[134]

As there were only 49 people in the smallest outcome group (late mortality), there was a limit to the number of terms that could be included in the multivariable model. If a covariate has n levels then it will require n-1 dummy variables in the model to account for each level of the variable. Therefore, if a covariate had multiple levels (e.g. Charlson score, with 9 levels), the levels were collapsed into clinically relevant groups to allow for inclusion of a greater number of covariates and interaction terms in the model. Age was kept as a continuous variable centered at age 18 years.

Time to appropriate antibiotic treatment was broken into 4 ordinal categories: <24 hours to appropriate treatment, 24-48 hours, >48 hours, or no appropriate treatment received. This was necessary in order to include patients who never received appropriate treatment, as they would have been lost if time was kept as a continuous variable.

Smoking status was broken into 2 nominal categories: current or former/never/missing smoker, as this was the only relevant category difference seen in the stratified analysis. The unknown/missing smoking status risk was not significantly different from former and never smokers in the stratified analysis; therefore, it was included with former/never smokers in the multinomial analysis.

Primary diagnosis was broken into 3 ordinal categories: meningitis, pneumonia/empyema, or bacteraemia/other invasive. These groups were chosen based on severity of disease, with meningitis being the most severe diagnosis and bacteraemia/other invasive being considered the least severe and acting as baseline.

The Charlson comorbidity index was classified as a Charlson of 0, 1, or ≥2 *a priori*. This variable was difficult to categorize because a patient can receive a certain score by having multiple diseases or by having one very severe disease that receives a high score

in the Charlson weighting system. In addition, the incremental difference between 1 and 2 in the Charlson scale is not equivalent to the difference between 5 and 6 and so on. Therefore, it could not be considered as a continuous variable with equally sized increments. The breakdown for Charlson was based on the distribution of the Charlson index in the data and the clinical intuition that those with a Charlson of 0 are similar to each other, those with a Charlson of 1 are similar, and those with a Charlson of ≥2 are more similar to each other than they are to those with 0 or 1. There were very few people with a Charlson comorbidity index >3; therefore, it seemed clinically appropriate to classify the groups this way. The Charlson of 0 was considered baseline for this variable.

3.6.3.3 Interaction Terms

When the effect of one variable on the dependent variable (mortality) is different for different values of another variable it is known as interaction. This may result in a synergistic effect, where two variables together increase the risk more than each would alone. Or it may cause an antagonistic effect, where there a smaller risk seen together than alone. Multivariable analysis allows for inclusion of interaction terms for measuring effect modification, which accounts for a covariate modifying the relationship between the dependent variable (outcome) and the other independent variable (exposure). Only pair-wise interaction terms were included in this project. Pair-wise interactions are interaction terms that account for interactions between just two variables (e.g. severity and primary diagnosis) as opposed to interactions between three or more covariates.

Survivors were the baseline group, so early and late mortality are compared to survival when interpreting the multinomial model. Inclusion of all pair-wise interaction terms between the 6 covariates to look for interactions between confounders was not possible due to the limited sample size. Instead, a more informed model was considered where only biologically plausible and clinically relevant interaction terms were considered. In the informed model interaction terms were included for all of the 6 main covariates as effect modifiers, as well as interaction terms for possible joint confounding from age and

primary diagnosis, Charlson index and time to appropriate treatment, primary diagnosis and time to appropriate treatment, and age and time to appropriate treatment. These “joint confounding” terms also allow for investigation of whether these covariates may modify each other as independent risk factors. For example, if primary diagnosis is an independent risk factor it may be modified by time to appropriate antibiotic treatment.

3.6.3.4 Backward Elimination and Forward Selection

A final model was achieved using backward elimination from the initial informed model. Any terms that were significant at an alpha level of 0.05 were kept in the model. If a term was clinically relevant or near significance (alpha level up to 0.10) it was also kept in order to maintain a fully adjusted model. After removal of each non-significant term, each model was compared to the previous model and the initial model using a likelihood ratio test. If there was not significant difference in how the model fit the data according to the likelihood ratio test (alpha set at 0.05), the model was kept and further terms were removed until a final model was chosen. Any terms that were independent risk factors or confounders were kept in the model, as the purpose was to better understand the overall picture of death due to IPD.

3.6.3.5 Full Multinomial Models

E=Severity

D=Primary Diagnosis

M=Gender

S=Smoking Status

C=Charlson index

T=Time to Appropriate Antibiotics

A=Age (continuous)

Late mortality (L) compared to survival (S - baseline)

$$\log\left(\frac{P_L}{P_S}\right) = \beta_{L1}E + \beta_{L2}D + \beta_{L3}M + \beta_{L4}S + \beta_{L5}C + \beta_{L6}T + \beta_{L7}A + \beta_{L8}ED + \beta_{L9}EM + \beta_{L10}ES + \beta_{L11}EC + \beta_{L12}ET + \beta_{L13}EA + \beta_{L14}DT + \beta_{L15}DA + \beta_{L16}CT + \beta_{L17}AT$$

Early mortality (E) compared to survival (S - baseline)

$$\log\left(\frac{P_E}{P_S}\right) = \beta_{E1}E + \beta_{E2}D + \beta_{E3}M + \beta_{E4}S + \beta_{E5}C + \beta_{E6}T + \beta_{E7}A + \beta_{E8}ED + \beta_{E9}EM + \beta_{E10}ES + \beta_{E11}EC + \beta_{E12}ET + \beta_{E13}EA + \beta_{E14}DT + \beta_{E15}DA + \beta_{E16}CT + \beta_{E17}AT$$

The full models did not achieve convergence, likely because they included more variables than the data could handle. When the apparently non-significant interaction term for time to appropriate treatment and age was removed, the next model achieved convergence.

Forward selection was also attempted; however, due to a small amount of data, all terms changed the estimates for severity by at least 15%, making this method difficult to assess. As a result, the final models used were chosen through backward elimination starting from the informed models. All analyses were performed using STATA Intercooled statistical software, version 11.0.

3.7 Ethics

CASPER has ethics approval from the Conjoint Health Research Ethics Board (CHREB) of the University of Calgary and Calgary Zone of Alberta Health Services for data collection and analysis. This project received ethics approval from the CHREB as a CASPER sub-study.

Chapter Four: Results

4.1 Descriptive Analysis

4.1.1 Patient Characteristics

There were a total of 1008 episodes of IPD in 973 Calgary adults from 2000 to 2009. Twenty-five eligible people did not have full reviews done; however, most of these patients still had information on demographics and outcome available. Seven patients refused participation in the study and 3 reviews were not done due to language barriers with the patient and next of kin that precluded consent. Only basic demographic information was available for these 10 patients, although outcome information was collected for the 3 patients with language barriers. The remaining patients had basic information from notifiable disease reports and lab reports. The patients without reviews were included in descriptive analyses where information was available. Nine-hundred and eighty-three (97.5%) of 1008 episodes had complete information and were included in the multivariable analysis.

The mean age of the sample was 54.6 (SD 17.86). The largest proportion of episodes (74.8%) had pneumonia as the primary diagnosis. Meningitis accounted for 4.1% of the diagnoses.

Table 4. Characteristics of Population

Characteristic	N (% of total)
Overall population N	1008
Population with full reviews	983 (97.5%)
Age group (N=1008)	
18-64 years	730 (72.4)
65-84 years	226 (22.4)
85+ years	52 (5.2)
Gender (N=1008)	
Male	586 (58.1)
Female	422 (41.9)
Comorbidities (N=983)	
Charlson comorbidity index=0	372 (37.8)
Charlson comorbidity index=1	263 (26.7)
Charlson comorbidity index ≥2	348 (35.4)
Serotype Causing Infection (N=995)	
PCV7 Serotype	260 (26.1)
PPV-23 Serotype (not in PCV7)	601 (60.4)
Non-Vaccine Serotype	134 (13.5)

Figure 6. Age Distribution of Entire Calgary Adult Population with IPD

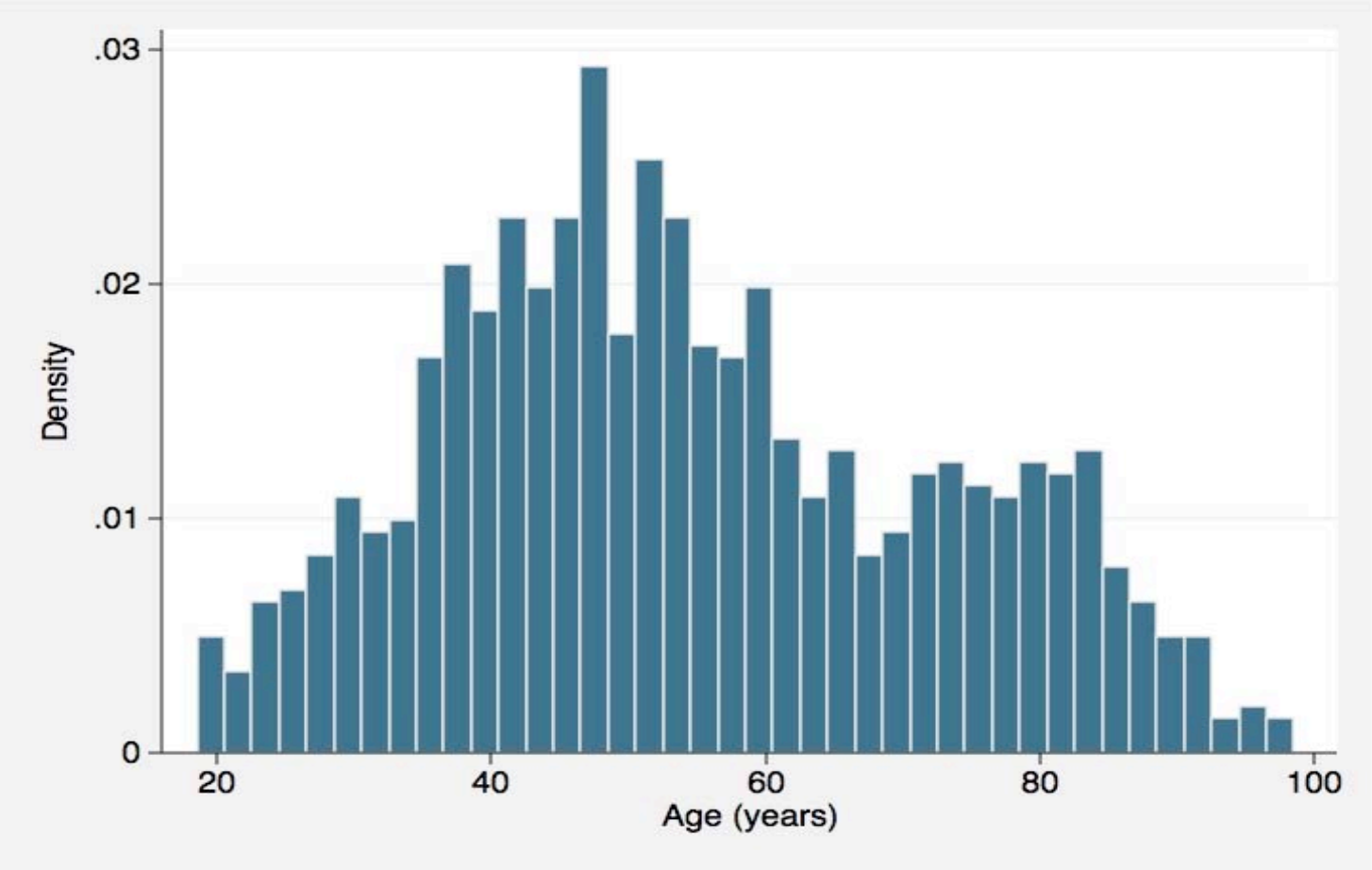


Table 5. Antibiotic Resistance Levels*

Antibiotic	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Penicillin	1001 (99.8)	1 (0.1)	1 (0.1)
Cefotaxime	1002 (99.9)	1 (0.1)	0 (0.0)
Ceftriaxone	1002 (99.9)	0 (0.0)	1 (0.1)
Erythromycin	920 (91.8)	11 (1.1)	71 (7.1)
Levofloxacin	1001 (99.8)	0 (0.0)	2 (0.2)
Meropenem	994 (99.1)	5 (0.5)	4 (0.4)
TMP/SMX	719 (71.8)	237 (23.7)	46 (4.5)

*Calculated using new CLSI MIC cut-offs as of 2011.[55] If patient had meningitis, meningitis cut-off was used. Otherwise non-meningitis cut-offs were used.

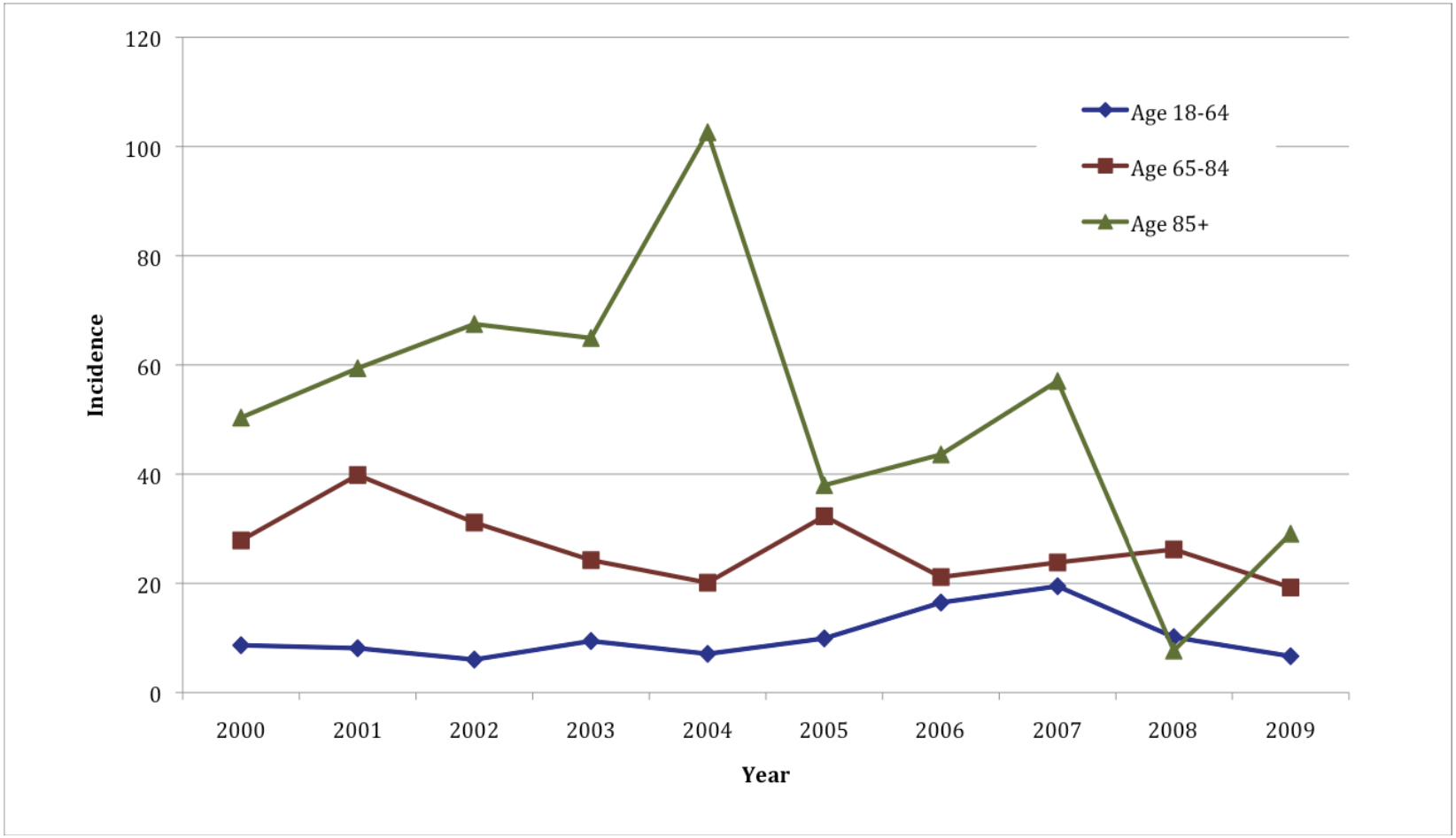
4.1.2 Incidence Rates of IPD

The trends for incidence of IPD in patients age 85+ fluctuate markedly. However, there is a low number of cases each year in this age group, causing variation to be more evident. Incidence of IPD in age 65-84 appears to decrease following introduction of the PCV7 vaccine in 2002. The incidence of IPD in 18-64 year olds does not appear to be affected by the introduction of the PCV7 vaccine. Serotype 8 and serotype 5 outbreaks in middle-aged adults in 2005 and 2006/2007,[22] respectively, countered PCV7 vaccine herd effects seen in these data.

Table 6. Incidence of IPD per 100,000 People per Year by Age Group

Age group	Year									
	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
18-64	8.7	8.1	6.0	9.4	7.1	9.9	16.5	19.5	10.1	6.6
65-84	27.9	39.8	31.1	24.2	20.1	32.3	21.1	23.8	26.2	19.2
85+	50.3	59.4	67.5	64.9	102.6	38.0	43.8	57.1	7.7	29.1

Figure 7. Trends in Incidence of IPD per 100,000 People from 2000-2009 by Age Groups



4.1.3 Case Fatality Rates

Of the 1001 patients with known outcomes, 119 patients died of IPD within 30 days of presentation giving an overall case fatality rate of 11.8%. Of the 119 patients who died, 58.8% died less than 5 days after presentation (early mortality).

Table 7. Age and Gender Specific Case Fatality Rates

Characteristic	Case Fatality Rate (%)
Age Group	
18-64 years	7.7
N=724	
65-84 years	21.3
N=225	
85+ years	28.8
N=52	
Gender	
Male	9.3
N=582	
Female	15.5
N=419	

4.1.4 Serotype Frequencies

PCV7 serotypes accounted for 26% of 995 IPD cases with serotypes available and 27.7% of 119 deaths from 2000 to 2009 and PPV-23 serotypes accounted for 86.5% of cases and 83.2% of the deaths from 2000 to 2009. The PCV7 vaccine was introduced for routine use in infants in 2002, leading to a decline in PCV7 serotype prevalence in IPD cases at all ages. The change in serotype distribution of this sample correlates with the introduction of the PCV7 vaccine. In the year 2000, the PCV7 vaccine serotypes accounted for 59% of 79 IPD cases and 60% of the 10 deaths. In 2009, PCV7 serotypes accounted for only 13% of 72 IPD cases and 22.2% of 8 deaths. PCV13 serotypes accounted for 61.1% of the cases of IPD in 2009 and 50% of the deaths. Figure 7 shows the change in serotype distribution from 2000-2009.

The most common serotype causing disease in this sample of 995 Calgary adults was serotype 5 (17.36%). The other most common serotypes were 8 (7.74%), 3 (7.4%), 4 (7.34%), and 22F (6.55%). In 2005 and early 2006 there was a serotype 8 outbreak primarily in homeless adults aged 18-64 years.[22] Similarly, in 2006-2007 there was a large outbreak of serotype 5 in homeless adults in Calgary.[22] These outbreaks explain the increase in IPD due to non-PCV7 serotypes between 2004 and 2008 seen in figure 8. The highest percentage of deaths over the study period were due to serotype 3 (12.6%), 22F (10.1%), 4 (6.7%), 11A (6.7%), 6B (6.0%), 19F (6.0%). Proportions of IPD and death caused by each serotype are shown in table 8.

A variety of serotypes caused meningitis. Of the 41 meningitis cases, the largest proportion were caused by serotype 4 (14.6%) followed by serotypes 8 (9.8%), 23F (9.8%), and 3 (7.3%). Serotypes 6B, 18C, 19F, 11A, 19A, 6A, 34 each caused 2 (4.9%) cases of meningitis.

The largest proportion of pneumonia/empyema cases (N=837) were caused by serotype 5 (19.8%), which is related to the outbreak of serotype 5 among adults aged 18-64. Serotypes 3 and8 each caused 68 (8.0%) cases of pneumonia. Serotype 4 caused 62 (7.3%) cases, and 22F caused 54 (6.4%) pneumonia cases.

Bacteremia/other invasive cases (N=117) were fairly evenly distributed amongst several serotypes. Serotype 22F caused the most with 11 (9.0%) cases. Serotypes 6B, and 23F each caused 7 (5.7%) cases of bacteremia/other invasive. Serotypes 4, 5, and 8 each caused 6 (5.0%) cases, and serotypes 3, 11A and 6A each caused 5 (4.1%) cases.

Only column percents are presented in table 8 for the primary diagnoses. A hierarchy was used in choosing a primary diagnosis for each patient. For instance, one patient may have presented with meningitis, pneumonia, and bacteremia, but would be classified only as a meningitis case. Therefore, the methods used cannot speak to the proportions of each diagnosis a single serotype caused (row percents).

Figure 8. Serotype Distribution: PCV7 Serotypes Compared to Non-PCV7 Serotypes from 2000-2009

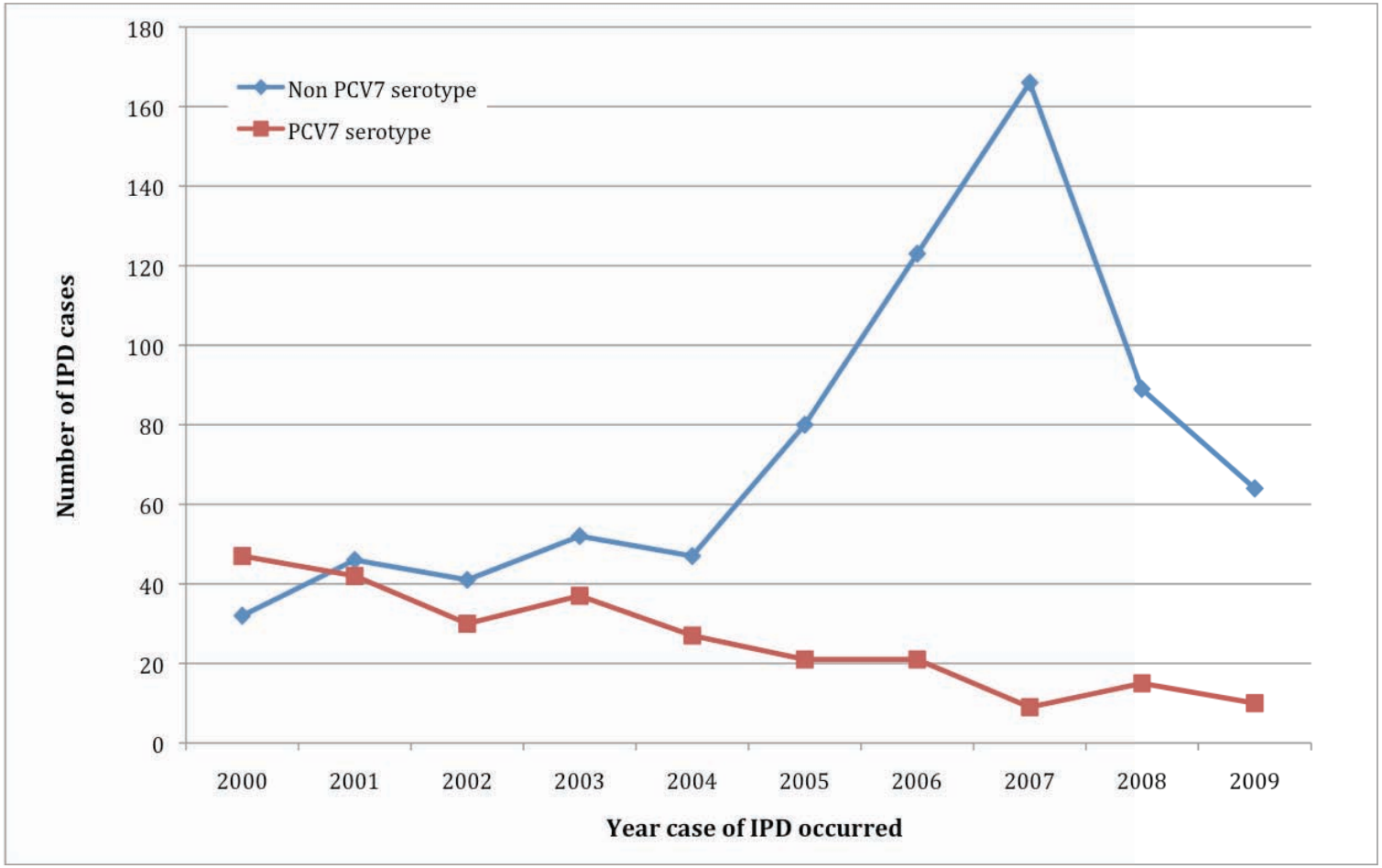


Table 8. Serotype Frequencies

	Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
PCV7 serotypes	4	74 (7.3)	8 (6.7)	6 (5.0)	62 (7.3)	6 (14.6)
	6B	32 (3.2)	7 (6.0)	7 (5.7)	23 (2.7)	2 (4.9)
	9V	29 (2.9)	0 (0.0)	3 (2.5)	26 (3.1)	0 (0.0)
	14	56 (5.6)	5 (4.2)	4 (3.3)	51 (6.0)	1 (2.4)
	18C	24 (2.4)	1 (0.8)	4 (3.3)	19 (2.2)	2 (4.9)
	19F	19 (1.9)	7 (6.0)	7 (5.7)	10 (1.2)	2 (4.9)
	23F	26 (2.6)	5 (4.2)	7 (5.7)	15 (1.8)	4 (9.8)
PPV-23 serotypes not in PCV7	1	13 (1.3)	0 (0.0)	1 (0.8)	12 (1.4)	0 (0.0)
	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
3	76 (7.4)	15 (12.6)	5 (4.1)	68 (8.0)	3 (7.3)
5	175 (17.4)	4 (3.4)	6 (5.0)	168 (19.8)	1 (2.4)
7F	34 (3.4)	3 (2.5)	0 (0.0)	33 (3.9)	1 (2.4)
8	78 (7.7)	5 (4.2)	6 (5.0)	68 (8.0)	4 (9.8)
9N	34 (3.4)	5 (4.2)	4 (3.3)	29 (3.4)	1 (2.4)
10A	6 (0.6)	1 (0.8)	3 (2.5)	2 (0.2)	1 (2.4)
11A	24 (2.4)	8 (6.7)	5 (4.1)	17 (2.0)	2 (4.9)
12F	34 (3.4)	3 (2.5)	3 (2.5)	31 (3.7)	0 (0.0)
15B	6 (0.6)	2 (1.7)	3 (2.5)	3 (0.4)	0 (0.0)
17F	8 (0.8)	1 (0.8)	0 (0.0)	7 (0.8)	1 (2.4)

	Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
	19A	29 (2.9)	4 (3.4)	4 (3.3)	23 (2.7)	2 (4.9)
	20	4 (0.4)	0 (0.0)	0 (0.0)	4 (0.5)	0 (0.0)
	22F	66 (6.6)	12 (10.1)	11 (9.0)	54 (6.4)	1 (2.4)
	33F	14 (1.4)	3 (2.5)	2 (1.7)	12 (1.4)	0 (0.0)
Non-vaccine serotypes	6A	33 (3.3)	5 (4.2)	5 (4.1)	26 (3.1)	2 (4.9)
	7C	2 (0.2)	1 (0.8)	1 (0.8)	1 (0.1)	0 (0.0)
	9L	4 (0.4)	0 (0.0)	1 (0.8)	3 (0.4)	0 (0.0)
	10F	2 (0.2)	0 (0.0)	0 (0.0)	2 (0.2)	0 (0.0)
	11B	5 (0.5)	0 (0.0)	0 (0.0)	5 (0.6)	0 (0.0)
	11C	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)

Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
11F	1 (0.1)	1 (0.8)	0 (0.0)	1 (0.1)	0 (0.0)
12A	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
13	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
15A	5 (0.5)	1 (0.8)	2 (1.7)	3 (0.4)	0 (0.0)
15C	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
16F	14 (1.4)	4 (3.4)	1 (0.8)	12 (1.4)	1 (2.4)
18B	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
21	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
22A	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
23A	6 (0.6)	1 (0.8)	3 (2.5)	3 (0.4)	0 (0.0)

Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
23B	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
28A	2 (0.2)	0 (0.0)	0 (0.0)	1 (0.1)	1 (2.4)
29	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)
31	14 (1.4)	1 (0.8)	0 (0.0)	14 (1.7)	0 (0.0)
33A	4 (0.4)	0 (0.0)	0 (0.0)	4 (0.5)	0 (0.0)
34	6 (0.6)	3 (2.5)	3 (2.5)	2 (0.2)	2 (4.9)
35A	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
35B	10 (1.0)	2 (1.7)	0 (0.0)	9 (1.2)	1 (2.4)
35C	1 (0.1)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
35F	6 (0.6)	1 (0.8)	1 (0.8)	5 (0.6)	0 (0.0)

Serotype	Overall Frequency N (%)	Mortality N (%)	Bacteremia/ Other Invasive N (%)	Pneumonia/ Empyema N (%)	Meningitis N (%)
38	8 (0.8)	0 (0.0)	3 (2.5)	5 (0.6)	0 (0.0)
Not available	2 (0.2)	0 (0.0)	1 (0.8)	1 (0.1)	0 (0.0)
Not viable/Not Frozen	7 (0.7)	0 (0.0)	2 (1.7)	5 (0.6)	0 (0.0)
Not typeable	4 (0.5)	0 (0.0)	1 (0.8)	3 (0.4)	0 (0.0)
Total	1008 (100.0)	119 (100.0)	121 (100.0)	847 (100.0)	41 (100.0)

4.1.5 Comparison of Proportions and Means

The mean was used as a measure of central tendency for age because the skew was low, and t-tests are robust to slightly skewed data. The median was only 1.2 years different from the mean for those who died and 2.2 years different from the mean for those who survived. The range of ages was 18.5-97.8 for those who survived and 24.1-97.5 for those who died; therefore, the differences between the mean and median were not considered important enough to require using the median as the measure of central tendency.

Table 9 shows means and Table 10 shows proportions for all-cause mortality compared to survivors. Patients who died had a higher mean age than those who survived ($P<0.001$). The results showed a greater proportion of females died than males ($P=0.0027$). There was a higher proportion of patients with a Charlson comorbidity index of ≥ 1 who died than a Charlson score of 0 ($P<0.001$). A greater proportion of deaths were seen among patients with meningitis than with non-meningitis IPD ($P<0.001$). Patients admitted to ICU had a higher proportion of death than those not admitted to ICU ($P<0.001$). Similarly, there was a higher proportion of deaths among patients with mechanical ventilation than those who did not require mechanical ventilation ($P<0.001$).

Table 11 shows comparisons of means and table 12 shows comparisons of proportions for early mortality compared to late mortality. For the early and late mortality comparison, only those who died were included, and one person was missing time from presentation to death; therefore, 118 episodes were included. Only the mean time to appropriate treatment was significant ($P=0.009$). Patients who died early (7.3 hours (SD:10.0)) had a lower mean time to treatment than those who died late (21.6 hours (SD:39.1)).

Table 9. Comparison of Means of Continuous Risk Factors for Survivors and Non-Survivors of IPD

Characteristic	Survivors Mean (SD)	All Cause Mortality Mean (SD)	Difference	95% CI of Difference	P-value*
Age (N=1001)	53.0 years (17.5)	65.7 years (17.1)	-12.7	-16.0 to -9.3	<0.001
Time to Appropriate Antibiotic Treatment (hours) (N=983)	12.0 (40.9)	13.1 (27.0)	-1.1	-9.3 to 7.0	0.7855

*Significant at an alpha level of 0.05

Table 10. Comparison of Proportions of Deaths by Categorical Risk Factors in Patients with IPD

Characteristic	Categories	All Cause Mortality N (%)	Difference (%)	95% CI of Difference	P-value*
ICU Admission (N=1001)	Admitted	63 (26.5)	-19.1	-25.0 to -13.2	<0.001
	Not admitted	56 (7.3)			
Mechanical Ventilation (N=1001)	Mechanical ventilation	63 (32.5)	-25.5	-32.4 to -18.7	<0.001
	No mechanical ventilation	56 (6.9)			
Gender (N=1001)	Male	54 (9.3)	6.2	2.0 to 10.4	0.0026
	Female	65 (15.5)			

Characteristic	Categories	All Cause Mortality N (%)	Difference (%)	95% CI of Difference	P-value*
Comorbidity	Charlson comorbidity index=0	27 (7.0)	-8.0	-11.8 to -4.2	<0.001
(N=1001)	Charlson comorbidity index≥1	92 (14.7)			
Diagnosis	Meningitis	16 (39.0)	-28.3	-43.3 to -13.2	<0.001
(N=1001)	Non-meningitis	103 (10.7)			
	Empyema	11 (12.2)			
	Pneumonia	74 (9.8)			
	Other Invasive	1 (5.0)			

Characteristic	Categories	All Cause Mortality N (%)	Difference (%)	95% CI of Difference	P-value*
	Bacteraemia (without focus)	17 (17.7)			
Smoking Status	Never Smoker	23 (12.5)			
			3.2	-2.1 to 8.5	0.1981
(N=888)	Ever Smoker	66 (9.3)			
	Former smoker	20 (10.3)			
	Current smoker	46 (9.0)			
	Unknown	30 (26.5)			

*Significant at an alpha level of 0.05

Table 11. Means of Continuous Risk Factors for Early Mortality Compared to Late Mortality in Patients with IPD

Characteristic	Late mortality Mean (SD)	Early mortality Mean (SD)	Difference	95% CI of Difference	P-value*
Age (N=118)	65.4 (17.2)	65.9 (17.2)	-0.49	-6.8 to 5.8	0.8784
Time to Appropriate Antibiotic Treatment (hours) (N=117)	20.8 (38.4)	7.3 (10.0)	13.5	3.1-24.0	0.0115

*Significant at an alpha level of 0.05

Table 12. Comparison of Proportions of Early Mortality by Categorical Risk Factors in Patients with IPD (N=118, Survivors not included)

Characteristic	Categories	Early mortality N (%)	Difference (%)	95% CI of Difference	P-value*
ICU Admission (N=118)	Admitted to ICU	34 (54.0)	10.3	-7.3 to 27.9	0.2537
	Not Admitted to ICU	36 (64.3)			
Mechanical Ventilation (N=118)	Mechanical Ventilation	35 (55.6)	6.9	-10.7 to 24.6	0.4423
	No mechanical ventilation	35 (62.5)			
Gender (N=118)	Male	31 (57.4)	2.6	-15.2 to 20.4	0.7748
	Female	39 (60.0)			

Characteristic	Categories	Early mortality N (%)	Difference (%)	95% CI of Difference	P-value*
Comorbidity	Charlson comorbidity index=0	12 (44.4)			
			-18.6	-39.8 to 2.6	0.0842
(N=118)	Charlson comorbidity index ≥1	58 (63.0)			
Diagnosis	Meningitis	8 (50.0)			
			10.2	-16.1 to 36.5	0.4408
(N=118)	Non-meningitis	62 (60.2)			
	Empyema	7 (63.6)			
	Pneumonia	42 (56.8)			
	Other Invasive	1 (100.0)			

Characteristic	Categories	Early mortality N (%)	Difference (%)	95% CI of Difference	P-value*
	Bacteraemia (without focus)	12 (70.6)			
Smoking Status	Never Smoker	13 (56.5)			
			2.0	-21.6 to 25.5	0.8697
(N=89)	Ever Smoker	36 (54.5)			
	Former Smoker	13 (65.0)			
	Current Smoker	23 (50.0)			
	Unknown	21 (70.0)			

*Significant at an alpha level of 0.05

In tables 10 and 12 smoking status and primary diagnosis are broken down into polytomous categories, meaning they have multiple levels (e.g. the 5 levels of primary diagnosis are meningitis, empyema, bacteremia, pneumonia, and other invasive). The purpose of the comparisons of proportions is only to describe the data; therefore, where applicable, polytomous categories were collapsed into two clinically relevant categories to allow for simple comparisons. The differences and p-values for those two categories are reported in table 10 and table 12, and only the proportions are reported for the breakdown of the polytomous form of the variable.

Inferences from the tests of proportions and their p-values can only be interpreted as individual comparisons of the proportions for the risk factor at hand, while ignoring all other possible existing associations. It would be incorrect to make simultaneous inferences from these individual tests. The multinomial regression produces results for each variable in the presence of all other variables included in the model; therefore, allowing for simultaneous inferences from all regression results. Main inferences for this study were made from the multinomial regression results.

Table 13. Proportions of Charlson Comorbidity Index in Survivors Compared to All-Cause Mortality

Charlson comorbidity index	Survivors N (%)	All Cause Mortality N (%)
0	360 (40.8)	27 (22.7)
1	238 (27.0)	26 (21.8)
2	111 (12.6)	24 (20.2)
3	74 (8.4)	17 (14.3)
4	25 (2.8)	10 (8.4)
5	11 (1.3)	3 (2.5)
6	25 (2.8)	4 (3.4)
7	23 (2.6)	3 (2.5)
8	7 (0.8)	4 (3.4)
9	8 (0.9)	1 (0.8)
Total	882 (100%)	119 (100%)

Chi2: P<0.001

The Chi2 results of <0.001 indicates that somewhere in the comparisons of the two groups, there is at least one difference between the groups in the proportion of people with a specific Charlson comorbidity index. Charlson index was grouped into relevant score categories for further analysis to allow for more useful interpretations.

4.1.6 Stratified Analyses

The crude estimate indicates that patients presenting with severe disease have 4.3 (95% CI: 3.0-5.9) times the risk of death compared to those with less severe disease. This difference in death between severity groups (risk ratio) appears to be a more dramatic difference for those with a Charlson of 0 (RR: 10.3 (95% CI: 4.5-23.5)), and for those who are young (RR: 9.4 (95% CI: 5.2-17.0)). The risk ratio estimates between each age group and between each Charlson index group are not statistically different (though they are statistically significant in that severity increases risk of death). Although the risk ratio estimates for each age category (18-64, 65-84, 85+) are not significantly different from one another statistically, the differences between these age categories may be of clinical interest. Therefore, age is reported as a clinically relevant effect modifier (a different risk ratio for each category of age) in table 14. Similarly, the three categories of Charlson comorbidity index are not statistically different, but may be different enough to be clinically relevant and are reported separately in table 14. Smoking acts as an effect modifier of the relationship between disease severity and risk of death, with higher risk of death among current smokers with severe disease compared to former and never smokers with severe disease (table 14). Time to appropriate antibiotic treatment, gender, and primary diagnosis did not modify or confound the relationship between severe disease and mortality according to stratified analysis. Only results that were statistically significant or potentially clinically relevant are reported in table 14. Statistically, age groups and Charlson comorbidity index groups should be reported as the crude risk ratio because they did not confound or modify. However, the separate risk ratios were different enough that there may be clinically relevant effect modification even if statistically significant differences were not observed between the groups. As a result, the separate estimates are reported in table 14.

In stratified analysis age, gender, Charlson index, smoking status, time to appropriate antibiotic treatment, and primary diagnosis did not modify or confound the relationship

between disease severity and risk of early mortality compared to late mortality among the 119 people who died. The crude risk ratio was appropriate to report for all of the stratifications, as none of the covariates appeared as effect modifiers or confounders of the relationship between early mortality and disease severity. The crude risk ratio estimate was 0.88 (0.65-1.17), which was not statistically different from the null value of 1, suggesting that there is no greater risk of early mortality than late mortality among those with severe disease compared to those with less severe disease.

Table 14. Stratified Analysis of Covariates Effect on the Relationship Between Severity of Disease and Risk of All-Cause Mortality

Covariate	Risk Ratio	95% Confidence Interval
Crude	**4.3***	**3.0-5.9**
Age		
18-64	9.4 §	5.2-17.0
65-84	3.0 §	1.9-4.8
85+	4.1 §	2.5-6.7
Charlson comorbidity index		
Charlson 0	10.3 §	4.5-23.5
Charlson 1	7.7 §	3.5-16.7
Charlson ≥2	2.2 §	1.4-3.3
Smoking Status		
Former/never smoker or missing	3.0*	2.0-4.5
Current smoker	8.9*	4.7-17.1

*Statistically significant
§Indicates result is statistically significant for more severe disease causing increased risk of death, but each level within the category is not statistically different from other levels, though may be clinically relevant differences; therefore, reported separately.

4.2 Multivariable Analyses

The multivariable analysis was performed using 983 of 1008 episodes (97.5%). These episodes had complete data for all the covariates entered in the multinomial model. The final models were achieved starting from the informed model and removing non-significant terms through backward elimination. Terms in the model that were non-significant (P-value of >0.10) were removed one or two at a time and the new model was compared to the previous model and the original model using a likelihood ratio test with a significance level set at 0.05. Terms that were non-significant and could be removed without significantly altering how the model explained the data were not confounders and were therefore unnecessary to adjust for in the multinomial model. Effect modification was assessed based on significance of interaction terms. Interaction terms with a p-value of <0.05 were considered significant and separate RRR estimates were reported for each level of the effect modifier.

4.2.1 Final Multinomial Models

E=Severity
D=Primary Diagnosis
M=Gender
C=Charlson index
T=Time to Appropriate Antibiotics
A=Age (continuous)

Late mortality compared to survival (baseline)

$$\log\left(\frac{P_L}{P_S}\right) = \beta_{L1}E + \beta_{L2}D + \beta_{L3}M + \beta_{L5}C + \beta_{L6}T + \beta_{L7}A + \beta_{L8}ED + \beta_{L11}EC + \beta_{L15}DA$$

Early mortality compared to survival (baseline)

$$\log\left(\frac{P_E}{P_S}\right) = \beta_{E1}E + \beta_{E2}D + \beta_{E3}M + \beta_{E5}C + \beta_{E6}T + \beta_{E7}A + \beta_{E8}ED + \beta_{E11}EC + \beta_{E15}DA$$

4.2.2 Results of Multinomial Logistic Regression Analysis

Table 15. Risk Factors Associated with Early and Late Mortality Compared to Survival in Patients with IPD in Calgary

Risk Factor	Relative Risk Ratio Late Mortality	95% CI	P-value	Relative Risk Ratio Early Mortality	95% CI	P-value
Disease Severity						
Less severe disease	N/A (modified by Charlson, see below)			Reference group		
More severe disease	N/A (modified by Charlson, see below)			32.4*	4.9-215.5	<0.001
Comorbidities						
Charlson comorbidity index = 0	N/A (modified by disease severity, see below)			Reference group		
Charlson comorbidity index = 1	N/A (modified by disease severity, see below)			2.4	0.6-9.5	0.215
Charlson comorbidity index ≥2	N/A (modified by disease severity, see below)			5.3*	1.5-18.8	0.01

Risk Factor	Relative Risk Ratio Late Mortality	95% CI	P-value	Relative Risk Ratio Early Mortality	95% CI	P-value
Comorbidities Modified by Severity						
Less severe disease and Charlson comorbidity index =0	Reference group			N/A (severity not modified by Charlson, see above)		
More severe disease and Charlson comorbidity index=0	21.5*^	1.1-440.4	0.046	N/A (see above)		
Less severe disease and Charlson comorbidity index=1	$1.2x10^{-6}$ ^	0-.§	0.983	N/A (see above)		
More severe disease and Charlson comorbidity index =1	17.4^	0.8-371.0	0.068	N/A (see above)		
Less severe disease and Charlson comorbidity index ≥2	6.4*^	1.4-29.5	0.017	N/A (see above)		
More severe disease and Charlson comorbidity index ≥2	19.8^	0.95-414.8	0.054	N/A (see above)		
5 year increase in Age	1.1	0.8-1.6	0.587	1.1	0.9-1.3	0.453

Risk Factor	Relative Risk Ratio Late Mortality	95% CI	P-value	Relative Risk Ratio Early Mortality	95% CI	P-value
Gender						
Female			Reference group			
Male	0.8	0.4-1.4	0.396	0.6	0.4-1.1	0.081
Primary Diagnosis						
Bacteremia/other invasive diagnosis			Reference group			
Meningitis diagnosis	3.9	0.02-747.4	0.612	3.4	0.1-103.8	0.477
Pneumonia/empyema diagnosis	0.4	0.01-23.4	0.662	0.1	0.01-1.13	0.06

Risk Factor	Relative Risk Ratio Late Mortality	95% CI	P-value	Relative Risk Ratio Early Mortality	95% CI	P-value
Time to receipt of appropriate antibiotic treatment						
<24 hours post-presentation			Reference group			
24-48hrs post-presentation	1.4	0.38-5.03	0.626	1.1	0.3-3.4	0.905
>48hrs post-presentation	4.7*	1.58-13.9	0.005	$1.6x10^{-7}$	0-.§	0.993
No receipt of appropriate antibiotics	1.89	0.51-6.96	0.341	3.5*	1.6-8.0	0.003

*: significant values of multivariable analysis at a significance level of 0.05

^:interaction term was significant indicating effect modification at a significance level of 0.1

§: 0-. Indicates that a zero cell was present in the calculation, no inferences can be made from these estimates. See discussion in text below.

In multinomial regression survivors were chosen for the baseline category; therefore, all relative risk ratios are in comparison to survivors. Age was centered at 18 years. All relative risk ratios in table 15 are the same for patients of all ages because the estimate for the change in relative risk for increase in age was not significant. Table 15 shows risk factors associated with late mortality and early mortality in patients with IPD. Smoking status was not statistically significant as an independent risk factor, a confounder, or an effect modifier; therefore, it was not included in the final model.

Patients with more severe IPD had increased risk of early death compared to survivors. A Charlson comorbidity index ≥2 modified the relationship between severity and late death. Patients with less severe IPD and a Charlson comorbidity index of ≥2 had 5.8 (95% CI: 1.2-26.8) times the risk of late death compared to survivors, while patients with severe IPD and Charlson comorbidity index of ≥2 had 19.8 (95% CI: 0.95-414.8) times the risk of late death compared to patients with a Charlson index of 0. Patients of both severity groups with a Charlson comorbidity index of ≥2 had increased risk of early death.

Patients receiving appropriate antibiotics more than 48 hours after presentation had increased risk of late death, while patients who never received appropriate antibiotics had increased risk of early death. Being female was nearly significant as a risk for early death (P=0.081). The relationship between early mortality and disease severity was modified by primary diagnosis. Pneumonia diagnosis was nearly significant for decreased risk of early death compared to bacteremia diagnosis among those with less severe disease (P=0.063), and the interaction term for modification of severity by pneumonia diagnosis was also close to significance.

There was a zero cell in the multinomial model for a Charlson index of 1 and late death. No one who died late had a Charlson index of 1 and less severe disease. Therefore, a RRR for late death cannot be estimated for patients with a Charlson index of 1 and less severe disease. Similarly, no one who died early received antibiotics greater

than 48 hours after presentation, resulting in zero cells. Again, no estimate of effect can be made from this RRR estimate.

Interestingly, age was not significantly associated with risk of early or late death. For every 5 year increase in age there was 1.1 (95% CI: 0.8-1.6) times increase in relative risk of late death, but this was not significant (P=0.587). Age was modified by primary diagnosis for early mortality.

Chapter Five: Discussion

5.1 Summary of Results

This study sample of 1008 patients with IPD was similar to previous studies. The largest number of cases was in the 18-64 age group; however, the highest case-fatality was seen in the over 85 age group (28.8%). Previous studies have shown men to be more at risk for IPD.[84, 87] Similarly, we found more males (58%) than females (42%) with IPD.

Antibiotic resistance to first line drugs for IPD such as penicillin, levofloxacin, and third generation cephalosporins was low (table 5). The highest resistance was to erythromycin (7.1%), and the highest intermediate resistance was with TMP/SMX (23.7%). Erythromycin is only considered to be effective as a second drug in dual therapy against *S. pneumoniae* and TMP/SMX is not an ideal antibiotic choice for *S. pneumoniae*. Overall, isolates from patients with IPD in Calgary showed low antibiotic resistance during the time period of 2000 to 2009. The penicillin resistance classification is based on the new cut-offs from the CLSI guidelines, which are higher than the previous cut-offs for non-meningitis IPD.[55] Using the old guidelines for penicillin non-susceptibility (susceptible ≤ 0.06, intermediate 0.12-1.0, resistant > 1.0), 0.4% are resistant to penicillin and 4.5% have intermediate resistance.[135] However, this is still relatively low compared to other places. In the USA in 1996, one study showed 14% of isolates were reported to have intermediate or full resistance to penicillin.[53]

In this study cohort, 995 of 1008 *S. pneumonia* strains had serotypes available (98.7%), and all serotypes were known for fatal cases. There were 49 different serotypes that caused IPD in the current study, though only 29 (59%) of these caused at least one death and only 14 caused 4 or more deaths. Those that were not serotyped were not available, not viable, or could not be typed. Among the episodes with serotypes available, 26% (260/995) were caused by a serotype that is included in the PCV7 vaccine and 86.5% (861/995) were caused by a serotype that is covered by the PPV-23 vaccine. Among those who died, 83.2% were infected by a PPV-23 serotype. These results suggest that use of the PPV-23 in Calgary may help to decrease IPD in adults, assuming

the vaccine is effective, which is a point of controversy.[67, 70] The PCV13 vaccine was not available until after the collection of this data (2010), but considering in 2009 that over half (61.1%) the cases of IPD and exactly half the deaths were caused by PCV13 vaccine serotypes, use of the PCV13 vaccine in adults may be an effective method of reducing some IPD and death in adults.

In the univariable tests of proportions and t-tests for measures of central tendency, risk factors for death included older age, female gender, presence of a comorbidity (Charlson index of ≥ 1), diagnosis of meningitis, admission to ICU, and mechanical ventilation. These results are similar to previously reported results with regards to descriptive analysis (Appendix A). However, because these are univariable tests, they are limited with regards to how the effect estimates can be interpreted and inferred.

In the univariable analyses comparing early and late deaths without survivors there were no statistically significant differences between early and late mortality with tests of proportions. The only significant difference between early and late mortality was in comparing mean time to antibiotic treatment. However, in the univariable analysis time to treatment was kept as a continuous variable, which resulted in those who never received appropriate antibiotic treatment being left out. In the multivariable analysis, time to antibiotic treatment was split into 4 categories, with one category including patients who never received appropriate antibiotics. In the multivariable analysis it was possible to compare 4 groups, whereas with tests of proportions 4 group comparisons must be made with adjustments. Therefore, for the purpose of the descriptive analysis this variable was tested as a continuous variable. Only the multivariable results should be interpreted for time to antibiotics, as the t-test was for the purpose of describing the data.

In the multinomial logistic regression the early deaths and late deaths were compared to survivors as a baseline group. Here differences could be seen in how early mortality compared to survivors and how late mortality compared to survivors with regards to risk factors.

Risk factors for early mortality relative to survival included a high Charlson comorbidity index, severe disease, and never receiving appropriate antibiotics for IPD. Gender was nearly significant, with male gender being protective against early death in IPD patients. Risk factors for late death included a high Charlson comorbidity index (≥2) among those with less severe disease, and not receiving appropriate antibiotics for IPD until more than 48 hours after presentation. The relationship between disease severity and late death was modified by Charlson index. In patients with less severe disease a Charlson index of ≥ 2 was a risk factor for late mortality. In patients with more severe disease at presentation and a Charlson index of ≥ 2, increased risk of late death was not quite significant, but the strength of the association suggests clinical relevance. Age, in itself, was not a significant risk factor for late nor early mortality.

5.2 Interpretation of Results

5.2.1 Serotype Frequency

There was a broad distribution of serotypes causing IPD in the present study. The most common serotype causing disease in Calgary adults was serotype 5. This is misleading of normal patterns in Calgary, as there was an outbreak of serotype 5 among homeless adults in 2006-2007.[22] A similar outbreak occurred in Vancouver, BC around the same time.[23] According to a study by Marrie et al., the most common serotypes causing IPD in adults 18 years and older across Alberta between 2000 and 2004 were serotype 4 and serotype 14.[126] This was prior to the serotype 5 and 8 outbreaks, and may be a better representation of which serotypes commonly cause IPD in Calgary adults. Serotype 3 caused the highest number of deaths in the current sample, which is similar to what was seen by Marrie et al.[126] A study in Spain showed that serotype 3 was most often associated with pneumococcal pneumonia presenting with shock, which may partly explain the high death rate.[136] Other less invasive serotypes will also lead to fatal cases of IPD. Deaths in such cases may be more likely due to the patient's condition, as

elderly people and patients with comorbidities are often at risk of opportunistic infections by these less invasive serotypes, but are also at a high risk of death due to their poor health. Therefore, without doing an analysis adjusted for age and comorbidities, we cannot infer further information from the frequencies of occurrence and mortality associated with particular serotypes in the present study. However, a more complex adjusted analysis, considering individual serotypes, may also be misleading due to the small number of cases associated with most individual serotypes, and so was not performed.

The serotypes most commonly causing meningitis in the adults in this sample - serotypes 4, 8 and 23F - are all included in the PPV-23 vaccine. Meningitis has a high rate of fatality in adults; therefore, vaccination may prevent a number of deaths due to meningitis. However, there were also several cases of meningitis caused by serotypes that are not a part of the PPV-23 or PCV7 vaccines. Serotypes 6A, 16F, 28A, 34, 35B each caused one case of meningitis in this study sample.

Eighty-six percent of the overall IPD cases in the current study were caused by serotypes in the PPV-23 vaccine. Therefore, assuming the PPV-23 vaccine is effective, our data supports the use of the vaccine for prevention of many cases of IPD in adults. However, there is controversy about the effectiveness of PPV-23.[67, 68, 70] There may be a need for a more effective vaccination that is focused on prevention of serotypes that are common in adults. The PCV7 vaccine, while effective at instilling immunity, may only prevent a quarter of the IPD cases that occur in adults in Calgary according to the current results. The serotypes in the PCV7 and PCV13 vaccines are more associated with pediatric infections, although a higher proportion of adult serotypes are included within PCV13, and this vaccine was recently licensed for use in adults.

5.2.2 Mortality Risk Factors

There were no statistically significant differences between early and late mortality when tests of proportions for each covariate were done. This suggests that the difference between early and late mortalities are not due to one or more specific risk factors, but rather presentation early and late in the course of disease. Those with early mortality may have presented with more advanced disease, and therefore died earlier.

In the multinomial logistic regression, the early deaths and late deaths were compared to survivors as a baseline group. Many of the results that were significant in the tests of proportions were no longer significant in the fully adjusted multinomial logistic regression model.

5.2.2.1 Gender

The descriptive analysis showed females to be at increased risk of all cause mortality compared to males. Although gender was not significant in the multivariable analysis, it remained close to significance, and with a larger sample size this result may have been statistically significant. One study by Vallès et al. in 2006 found increased risk of death among females relative to males hospitalized with pneumonia due to *S. pneumoniae* (OR: 9.1, 95% CI 1.3-61.2).[137] This study found that women seemed to be admitted to hospital less often, but those who are admitted present with more severe pneumonia. The authors suggested further research was required to determine reasons for this difference.

Several previous studies have shown male sex to be a risk factor for IPD,[84, 87] but only one paper from the mortality risk factor literature review showed male sex to be a risk factor for death due to IPD.[12] The increased risk of IPD in males has been suggested to be partly explained by the distribution of alcoholism and other risk factors in men.

5.2.2.2 Age and Comorbidities

Age was significant in the comparison of mean age for all-cause mortality to survival, and nearly significant as an effect modifier in the stratified analysis. However, as a continuous variable in the multinomial logistic regression, age lost significance and was not linearly associated with increased risk of early nor late mortality.

In the stratified analysis, a similar pattern was seen for both Charlson index levels and age groups, suggesting that the pattern could potentially be due to only one of these, as age and comorbidities are often associated. This study was fairly unique in that age was entered in the multivariable model as a continuous variable rather than dichotomized or divided into age groups. The benefit of keeping age as a continuous variable is that there is no loss of information. In contrast, the problem with keeping age as a continuous variable, is it assumes a linear relationship between age and mortality. This analysis only included adults, and in adults age likely does have some linearity in the relationship with mortality. When the relationship was graphed, it was not far from linear (data not shown). However, in order to ensure the lack of significance of age was not due to lack of linearity between age and mortality, the multivariable analysis was re-run with age dichotomized at the commonly used cut off of <65 or ≥ 65. The results showed an estimated RRR of 1.88 (95% CI: 0.42-8.38) for early mortality and an estimated RRR of 1.03 (95% CI: 0.08-13.94) for late mortality. Age was still not significant in this analysis, which suggests that use of a continuous variable did not cause the result.

In the model where age was kept as a continuous variable, the risk of early mortality per one year increase in age (note that in table 15 estimate is reported by 5 year age increase) was 1.02 (95% CI: 0.97-1.06) relative to survivors, and the increase in risk of late mortality per one year increase in age was 1.02 (95% CI: 0.95-1.10). Using these numbers, the RRR for a specific age can be calculated by taking 1.02 to the power of the age minus 18, because age was centered at 18 years. To calculate the difference in risk of death between two ages requires taking 1.02 to the power of the difference between the two ages. For example, the difference in risk between a 65-year-old and an 18-year-old

is 1.02 to the power of 47, which means 65-year-olds have 2.5 times the risk of early death (or late death) compared to 18-year-olds according to the current study findings. Therefore, it could be argued that although not statistically significant, there is clinical relevance to increasing age.

Furthermore, the *a priori* decision was to keep any term in the model that had a significant Wald test at an alpha level of 0.1. This was done because the model was intended to be exploratory and allow for understanding of what variables may influence outcomes. However, a greater number of terms in the model and a higher alpha level can allow for greater type 1 error. In *post-hoc* considerations the alpha level was lowered to 0.05 and the interaction term for the relationship of age and primary diagnosis with early mortality was removed (Wald test=0.08). Removing this interaction term caused age to become significant for both early and late mortality and only slightly changed all of the point estimates reported (indicating that it was not accounting for confounding); therefore it was appropriate to remove it from the model. The RRR estimate for age became 1.05 (95% CI: 1.03-1.07) increase in risk per 1 year increase in age for late mortality and early mortality when the interaction term was removed.

Another interaction term (primary diagnosis and severity for early mortality) with a Wald test p-value of 0.06 was kept in the model despite having a p-value greater than 0.05. Removal of this term changed the point estimate for the relationship between severity and early mortality by more than 10% suggesting that this term was accounting for "joint confounding."

Despite the discovery that a different model resulted in increasing age being significant, the conclusion remains that it is important to keep in mind that age is not the only important factor involved in risk of death due to IPD, and the current study suggests that it is less important than comorbidities and disease severity.

Previous studies have often found age to be a significant risk factor for mortality. However, many of these studies have only examined age using univariable analyses, and the results of the current study also found age to be significant with univariable

descriptive analysis.[43, 72, 85, 101, 114, 138] Further, studies that have examined age in a multivariable analysis generally looked at dichotomized age or age groups rather than age as a continuous variable.[86, 101, 139] Harboe et al., in studying the association of *S. pneumoniae* serotype with mortality using an adjusted logistic regression model, included age as a continuous variable and found that the odds of death significantly increased by 1.02 (95% CI 1.02-1.03) per 1 year increase in age for people ≥ 5 years of age.[12] The value of the odds ratio for age shown by Harboe et al. is the same per year increase as the RRR found in the current study. Harboe et al. had a very large sample (nearly 19,000 cases) which made it easier to find significance, even for small ORs. On the other hand, it is also possible that the analysis by Harboe et al. was incorrect in assuming a linear relationship between age and death across all ages, particularly when the incidence of all IPD is not linearly related to age since there are bimodal peaks of IPD in young children and the elderly. In the current study, because only adults were included, a linear relationship between age and death was a more reasonable assumption.

It has previously been reported by Garcia-Vidal et al. that 11% of patients presenting to hospital with pneumococcal pneumonia had septic shock, which has an important influence on prognosis.[136] This same study by Garcia-Vidal et al. found that patients with shock were significantly younger.[136] Patients who are younger may wait longer to seek medical care and present with more severe disease, including shock.

Currently, it is common for patients to be considered in terms of their "effective age" (i.e. the age that they are most similar to in terms of their health, a 50-year-old may have the health of an 80-year-old), rather than their chronological age. With large numbers of comorbidities, including malignancies and type II diabetes due to increasing prevalence of obesity, chronological age, in itself, may become less relevant. The results of this study indicate that comorbidities rather than age increase relative risk of early and late mortality. A Charlson comorbidity index of ≥2 increased the risk of early mortality among both severity groups (P=0.01) and increased the risk of late mortality in the less severe group (P=0.017). The relationship between disease severity and late death was

modified by a Charlson index ≥2, in that for patients with more severe disease the Charlson index was not statistically significant as a risk factor for late mortality. However, the RRR was large, and it was very close to significance. It is possible that patients who present late in the course of IPD and at a very severe stage of the disease are already at higher risk of death due to the severity of the infection and comorbidities become less relevant. Patients who present with less severe IPD may have a greater chance of survival through treatment of the infection. In the group with less severe disease, the presence of comorbidities had a statistically significant role in determining the patient's outcome. However, it is still clear that disease severity plays an important role in increasing risk of death regardless of Charlson index. While Charlsons index did modify the association between risk of death and disease severity, all patients with severe disease had significantly higher or nearly significantly higher risk of death relative to the baseline group. Patients with more severe disease also had much higher RRR even if these were not always statistically significant. Therefore, there may be clinical relevance to the RRR estimates, regardless of the results of hypothesis testing (p-values).

Previous studies have also shown comorbidities to be associated with mortality due to IPD, many of which used a severity score in the analysis. One study found a higher mean Charlson index to be associated with death,[16] though the use of a mean to analyze Charlson index is questionable, as it is an ordinal scale. Similar to the current study, Harboe et al. found a high Charlson index was a significant risk factor for mortality.[12] Grau et al. found comorbidities to be an independent predictor of mortality in HIV patients infected with IPD when adjusted for age, sex, CD4 cell count, alcohol abuse, shock at presentation, and leukopenia.[112] Age was not an independent risk factor in the study by Grau et al.[112]

5.2.2.3 Disease Severity

In the current study, increased disease severity was an important risk factor for early mortality. More severe disease was associated with risk of late mortality in patients with no comorbidity (Charlson index of 0), while it was not statistically significant in patients with comorbidities (Charlson index of 1 or ≥2), however the RRR estimates are high enough and close enough to significance that they should be considered clinically relevant.

It may be that patients who die early present later in the course of the disease, and, therefore, present with more severe disease. This was the initial hypothesis for this study, and the results support this. The current results also highlight that disease severity is an important risk factor in late death. This study was unable to measure the length of symptoms before presentation in order to evaluate whether more severe disease at presentation correlated with longer duration of symptoms prior to presentation. However, these results highlight the importance of preventive action such as vaccination in order to reduce both early and late mortality.

A study by Balakrishnan et al. evaluated what clinical and laboratory factors proved to be early predictors of mortality.[121] These signs included temperature below 37ºC, increased respiratory rate, and high arterial blood pH, among others.[121] These signs indicate a patient has more severe disease at presentation.

Several studies involving sepsis and pneumonia have shown that most deaths occur early.[7, 121-123, 125] It is likely that patients who present later in the disease course with a more severe clinical state, may die earlier. The current study found that people who died early had more severe disease, as increased disease severity was independently associated with risk of early death. This is another factor that may account for lack of significance with age. Young, healthy people may seek medical attention later in the disease course than patients who are elderly.

In the current study, there was also a significant association between early mortality and never receiving appropriate antibiotics, which may suggest that some patients who

died early presented too late in the course of disease for treatment to make a difference. These results indicate a need for a more effective vaccination for adults, preferably a vaccine that contains serotypes that cause higher rates of death in adults. Developing effective vaccinations and promoting the use of vaccines is important. The results from the current study especially support the need for an effective vaccine for patients with comorbid conditions, as they are at increased risk of both early and late mortality.

5.2.2.4 Appropriate Antibiotic Treatment

Previous research suggests that early mortality is related to delayed diagnosis and delayed initiation of appropriate antibiotic treatment.[124] The current study findings suggest that late deaths rather than early deaths are partly due to delayed initiation of appropriate antibiotic therapy, and early deaths are associated with no appropriate antibiotic therapy. Patients who present late in the course of IPD with very severe disease may not receive appropriate antibiotic therapy because it cannot be given in time or because the disease has already progressed so far that only palliative action is taken. This may, in part, explain the relationship between early mortality and no appropriate antibiotic therapy. If this is the case, either prevention of IPD or earlier presentation would be necessary to change the outcome. In some patients the disease may progress very rapidly, in which case prevention through vaccination would be the most effective option for decreasing mortality. However, the current PPV-23 vaccine is controversial and it should be reiterated that a number of studies suggest that it is not effective at preventing IPD.[67-71] There is a need for an effective adult vaccine against *S. pneumoniae* in order to reduce early deaths due to IPD.

Regarding antibiotic therapy, the greater concern is the delay in receipt of appropriate antibiotics that may be associated with increased risk of late death. It is important that effective empiric antibiotic therapy for a patient's syndrome be given as soon as possible after presentation to hospital. Once the causative agent of the infection is confirmed to be *S. pneumoniae*, antibiotic therapy should be continued or modified as needed to treat this

pathogen. There is controversy as to whether dual therapy is more effective for treatment of IPD, but the current recommendations still support dual therapy.[140] Resistant organisms may be an issue in treatment of some cases, as MIC testing may not be returned within 48 hours to advise physician antibiotic choices. However, due to the very low level of antibiotic resistance found in this study, it appears that in Calgary very few cases were affected by resistance during the study period. In most cases, timely receipt of any parenteral antibiotic appropriate for *S. pneumoniae* should be sufficient. Furthermore, previous research has not found a strong association between antibiotic resistant *S. pneumoniae* and increased risk of mortality.[14, 86, 111, 115, 117]

Previous studies have also suggested a relationship between timely receipt of appropriate treatment and death. One study found that receipt of inappropriate antibiotic therapy was associated with risk of mortality.[102] In a review of studies, Houck and Bratzler concluded that the earlier appropriate treatment is received for patient with CAP, the better the outcome.[141] They suggested that this was particularly true for elderly patients.[141] A Scandinavian study found a time period greater than 4 hours between admission and initial antibiotic treatment resulted in an increased risk of in hospital mortality (adjusted hazard ratio: 2.6, 95% CI 1.1-6.5).[142] Berjohn et al. showed that in adults with IPD who received antibiotics within 4 hours of presenting to hospital had decreased risk of mortality.[143] This result was adjusted for severity, but nothing else. The same study showed that receipt of antibiotics within 8 hours was no longer protective against death.[143] Odd ratios indicating risk of death increased with increasing two hour intervals from presentation to antibiotic receipt, though the results were not significant when broken down to this level of detail.[143]

5.2.2.5 Primary Diagnosis

In the univariable analysis, meningitis was significantly associated with mortality when compared to all other non-meningitis diagnoses. In the multivariable analysis, although meningitis diagnosis had a RRR > 1 relative to bacteremia/other invasive for both early

and late mortality models, neither result was significant. Interestingly, the one result that was close to significance (0.06) was pneumonia/empyema, which showed a lower relative risk of early mortality compared to bacteremia/other invasive. Although not statistically significant in this study, it is worth noting that bacteremia without focus has been suggested to have a higher association with morbidity and mortality than bacteremia that is associated with a focal infection such as pneumonia.[144] "Other invasive" disease was grouped with bacteremia in this study, which may have influenced this result. There were only 20 episodes classified as "other invasive", and only 1 of these died. In the descriptive analysis of the current study, among patients classified as only having bacteremia, 16.7% died, while lower proportions of mortality were seen with empyema (11.1%) and pneumonia (9.8%). As a result, it could be that pneumonia/empyema would have been significant as a clinical condition less likely to result in death if the "other invasive" group, which is arguably less severe, was not grouped together with the bacteremia without focus.

5.3 Strengths and Limitations

5.3.1 Strengths

Previous studies that have examined early mortality have looked at all-cause CAP or bacteremia. An Alberta, Canada study by Marrie et al. was the most similar to the current study; however, the analysis was not as comprehensive, fewer years were reported (2000 to 2004), and early and late mortality were examined in separate logistic regression models rather than the same model concurrently.[126] Therefore, the current study provides a unique perspective and more effective analysis.

Strengths of the current study include capturing all patients that present with IPD in the Calgary Health Zone, serotyping of all viable samples, and the use of multinomial analysis, which is a unique analysis to the current IPD literature. The CASPER network uses surveillance by the Calgary Laboratory Services to capture all cases of IPD. This

ensures a decreased risk of selection bias, as all infections that are serious enough to warrant a sterile sample will be identified and sent to CASPER. Because IPD is a relatively rare disease, having ten years worth of data allowed for a larger sample size and the ability to adjust for a greater number of variables in the multinomial model.

Some IPD surveillance programs do not serotype all isolates. Instead they choose a selection of samples to serotype and extrapolate the serotypes of the remaining samples from those that were serotyped. All CASPER episodes are sent for serotyping, and only the very small proportion that are not viable or non-typeable are excluded in analyses. This is beneficial in fully understanding the distribution of serotypes.

The use of multinomial analysis allowed for the break down of mortality into early and late, while preventing loss of information by analyzing all outcome levels in the same model. Early and late mortality were compared to survival to obtain relative risk ratios. These estimates allowed for understanding of what factors may increase the risk of early and late mortality relative to survival in IPD patients. Furthermore, the model was adjusted for confounding by variables chosen *a priori* based on previous literature and included interaction terms for effect modification and joint confounding. This analysis was more complete than most analyses seen in the previous literature regarding early mortality risk factors and risk factors for overall mortality.

Another strength of this study was the use of time to appropriate antibiotic therapy. This variable brought together a large amount of information by accounting for the antibiotic given as well as the time from presentation to receipt of the antibiotic, and it also made use of antibiotic resistance information. This variable allowed for effective analysis of a potentially modifiable factor.

5.3.2 Limitations

The most important limitation of this study is that the early and late mortality are measured from the time of patient presentation at a hospital. Patients will present at different stages in the illness; therefore, some may present at a more severe stage of the

disease. The true measure of early and late mortality from the time of infection is difficult to determine. Time of presentation was used as a surrogate time point. However, this is the reason for including disease severity as the primary exposure for the analysis, as it was hypothesized *a priori* that late presentation with severe disease may be the reason for early mortality. Furthermore, time of presentation is a very clinically relevant time point.

There are limitations to using ICU admission and mechanical ventilation as proxies for disease severity. Some elderly patients may have a care designation that causes them to not be admitted to the ICU despite having severe disease. We would misclassify these patients as having less severe disease, which could result in misclassification bias. If this occurred the bias would likely result in a decreased association between disease severity and early or late mortality causing the RRR to shift towards the null value of 1.0. Therefore, if anything, this would likely cause a dilution of the true effect, and in the worst case it may cause an effect in the opposite direction (protective effect). Because we did see an association of increased risk of mortality due to severe disease, it appears that any misclassification bias may have caused an underestimate of the effect, but this is less of a concern than a false estimate of an effect that is not actually there.

Calculation of the time to appropriate antibiotic covariate was limited in that it did not consider dose of antibiotic given. Resistance was assumed if the strain of *S. pneumoniae* showed intermediate resistance or full resistance. Therefore, some of the strains with intermediate resistance may have received appropriate antibiotics because a high enough dose of the antibiotic was given to overcome the resistance, but this would not have been captured. However, with the very small proportion of resistance to the primary antibiotics used for treatment of IPD (0.1-0.2%), this should not have influenced the findings. Erythromycin, which had the highest level of resistance was not considered to be appropriate as monotherapy for *S. pneumoniae* in the calculation of the appropriate antibiotic therapy. TMP/SMX was considered appropriate if given as IV therapy, therefore, this is the only antibiotic that may have been affected by a higher dose being

given. However, although TMP/SMX may be considered appropriate, it is not an ideal antibiotic for treatment of *S. pneumoniae.* It is known that there is high resistance in Calgary to TMP/SMX by *S. pneumoniae*; therefore, ideally, patients would not be put on this antibiotic when the causative agent is known.

The antibiotic therapy variable was also unable to compare mono versus dual antibiotic therapy. The literature is controversial as to whether dual therapy is a more effective method of treatment for IPD.[140] Feldman et al. reviewed 9 studies specific to mono versus dual therapy in patients with IPD, 5 studies were in favour of dual therapy[57-59, 145, 146] and 4 found no difference between mono and dual therapy.[60-62, 147] Among the studies in favour of dual therapy were three retrospective analyses, of which two were single center; one was a large, prospective, multicenter study; and one was a surveillance study. Among the studies that found no difference were two prospective, multicenter studies, and two retrospective, multicenter studies. This is an important debate, as the current recommendations are for dual therapy, but if two drugs actually have an antagonistic effect when given together, dual therapy may result in decreased effectiveness.

The most reliable study supporting dual therapy in IPD patients was conducted by Baddour et al. and was a multicenter, multinational study.[57] Baddour et al. found no difference between mono or dual therapy overall, but in patients who were critically ill according to their Pitt Bacteremia Score, their results suggested improved outcome with dual therapy.[57] However, it appears that these authors did not use effective multivariable analysis, they performed logistic regression separately adjusting for a single variable with each model rather than simultaneously adjusting for multiple factors at once.

The large multicenter, prospective study by Dwyer et al. compared beta-lactam monotherapy to beta-lactam plus macrolide dual therapy.[62] In a fully adjusted multivariable analysis they failed to find a difference in mortality between the two treatment groups.[62] However, it is unclear whether some patients classified as receiving a beta-lactam without a macrolide may have received a non-macrolide in dual-therapy. If

this were the case the effect estimate could have been diluted to the null.[62] Furthermore, although the data was prospectively collected, the original study was not designed to analyse antibiotic use in patients with IPD.[62]

Another multicenter, prospective study by Aspa et al. did not find any difference in effects of different antibiotic therapies for decreasing mortality due to IPD.[60] Beta lactam mono-therapy, beta-lactams in dual therapy with macrolides, macrolide monotherapy, and levofloxacin monotherapy were compared, as well as a group of other combination antibiotics. No differences were found in mortality, although Levofloxacin mono-therapy was close to significance as a protective factor (P=0.07).[60] However, these authors did not take into account the time from presentation to antibiotic receipt. Results from the current study suggest that the time to antibiotic receipt is important, provided the antibiotic given is appropriate. Several of the combinations examined by Aspa et al. would be appropriate for treatment of *S. pneumonia*, and not considering time may have caused a dilution of effects. If appropriate therapy was received, but not received in time, then the patient may have been classified inappropriately for the results to show an association with mortality.

Although CASPER is a prospective study, the chart reviews are done retrospectively, which may be a limitation, as certain information cannot always be confirmed once the patient has left the hospital. On the other hand, conducting a chart review while a patient is still in hospital may also result in missed information due to charts not yet being updated and complete.

Some patients may die before an interview can be performed by the CASPER study nurse. A chart review is conducted for these patients, and an interview with their next of kin if possible; however, the information obtained may be less complete. Missing data may result in information bias. Furthermore, information collected through interviews may be less reliable due to patient recall. With interviews there is always a potential for recall bias or reporting bias, which could also be a source of misclassification bias. These limitations should not have impacted the variables focused on in the current study

because the variables used were gathered from medical charts or laboratory data. All covariates used in this study came from the chart reviews, therefore recall bias should not have been an issue. The only concern may have been inconsistent reporting of factors such as smoking status and comorbidities in patient charts. However, because smoking and comorbidities are important in patient care and health, misclassification should have been low, and it should have been similar between patients who die and those who survive with regards to the variables focused on in the current study. Provided any misclassification bias was non-differential, it would have caused the effect estimate to go towards a null effect, causing an underestimate of associations. The variables that were included in the final model for mortality included variables that should have been more diligently recorded in charts: comorbidities, primary diagnosis, and antibiotic treatment. Age and gender were consistently reported and reliable variables. It is possible that smoking status, which did have a large amount of missing information, could have been non-significant and removed from the model due to the missing information. Therefore, the results from this study that suggest smoking was not a significant risk factor may not be reliable. Smoking may also be underreported due to people feeling that it is socially unacceptable, which may cause further misclassification of this variable.

Charlson score was used for adjustment of multiple co-morbidities simulataneously and included the most important co-morbidities related to IPD, which is a strength of this study. However, because the Charlson score does not account for every co-morbidity, it may have resulted in some patients who have a co-morbidity being classified with a Charlson of 0. Any co-morbidity could potentially increase a patient's risk of death due to IPD. For instance, Charlson score does not consider presence of asthma, but asthma has been suggested to be associated with risk of IPD;[148] therefore, asthma may also be associated with increased risk of death. As a result, misclassification of patients with comorbidities into the group with no co-morbidities may have actually caused the estimated association between comorbidities and IPD to be underestimated compared to the true association. This would be true both if the misclassification was non-differential

or differential. Differential misclassification would most likely be among patients who died and had a comorbidity but were misclassified as having no comorbidity. In this case the estimate would go towards the null.

Vaccination status could not be examined as a covariate, because it is based on patient recall and is missing for many patients and subject to recall bias among the rest. However, vaccination should have a greater affect on whether a patient develops invasive disease in the first place rather than whether they die. Furthermore, if this factor was confounding the relationship between disease severity and death, it would likely cause a dilution of effects because presumably it would cause a decrease in disease severity and decrease in death. Therefore, since severity was significant in the multivariable analysis, this may actually be an underestimate among unvaccinated people. Patients with comorbidities may have higher rates of vaccination. If this were the case, then, again, we may see a dilution of effects, and the increased risk among patients with a high Charlson comorbidity index may actually be an underestimate. Furthermore, the PPV-23 vaccine is controversial with regards to its effectiveness, particularly among people with comorbidities; therefore, it may not be a relevant factor to consider. The PPV-23 is also not consistently given in Calgary, and it is likely that few people were vaccinated. The most likely people to have received the vaccination are those who had a similar infection previously. However, according to an Alberta study, the PPV-23 vaccine is ineffective at preventing long term morbidity and mortality in patients who have previously been infected with *S. pneumoniae*.[70]

The CASPER data only includes information for patients up to 30 days after presentation to hospital; therefore, some deaths that occur more than 30 days after presentation may have been missed. However, Feikin et al. observed that only 4% of patients with pneumonia died more than 30 days after presentation,[86] indicating that most deaths from IPD should have been captured despite censoring data at 30 days.

5.4 Conclusions

Comorbidities increased risk of both early and late death, while age, in itself, was not associated with mortality in the initial multivariable analysis.

The results from the current study indicate a deficiency in initial treatment of invasive infections cause by *S. pneumoniae*, and an association with increased risk of late death. This may be a modifiable factor; some late deaths may be preventable with appropriate treatment within the first 24 hours after presentation. The association between early death and no appropriate antibiotic treatment suggests that many patients who die early may be presenting at a palliative stage of the disease when antibiotic therapy is not effective. Death due to IPD often occurs before therapy can make a difference in the disease course, which has been reported since 1964 in a study by Austrian and Gold.[7]

Current research does not support PPV-23 as an effective vaccine, particularly among patients with comorbidities. The PCV7, although more immunogenic, does not contain the most important serotypes in Calgary adults. The recently licensed PCV13 will provide better coverage for adults, but according to the results from the current study, over 50% of cases and deaths are due to non-PCV13 serotypes. Similar to previous literature, the results of this study indicate a need for a more effective vaccine against the primarily adult serotypes to prevent IPD in these high risk patients in Alberta.[16]

5.5 Future Research

There is currently very little research into early mortality in patients with IPD. Although IPD is a relatively rare disease, *S. pneumoniae* continues to be the most common cause of both invasive CAP and non-invasive CAP, which is much more common. Cases of rapid onset CAP continue to cause death.

This study was the first to examine early and late morality in a multinomial model, which allowed for less loss of information by allowing all levels of outcome to be included in one model. A similar analysis performed in a larger population may be beneficial to confirm the results found in the current study. A larger population may

allow for a large enough sample size to observe significant results for some of the near significant factors in the current study, or may confirm the lack of significance.

Primary diagnosis differences were not significant in the multinomial analysis, which is surprising, as meningitis is often associated with increased risk of death. Meningitis only occurred in 41 people in the current sample; therefore, a similar analysis in a larger population may allow for the power to detect differences that may occur between the different primary diagnoses. It may be beneficial to include data from other cities in Canada and to run these analyses on a larger sample size to gain greater precision.

Because recent research does not appear to support use of the PPV-23 vaccine,[67-71] the current results showing an association between death and lack of appropriate antibiotic therapy and delay in appropriate therapy, further indicate a need for an effective vaccine against *S. pneumonia* for adults. The current research supports a need for prevention more than treatment of IPD, as early deaths may not be preventable by treatment alone. For some patients presenting at a severe stage of IPD antibiotic therapy may be too late.

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APPENDIX A: LITERATURE SEARCH – RISK FACTORS FOR MORTALITY IN PATIENTS WITH IPD (SEARCH PERFORMED MARCH 23, 2011)

A.1. Literature Search – Description of Literature on Risk Factors for Mortality Due to IPD

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
Afessa et al. (1995) [114]	United States (USA)	Retrospective Cohort	293	Age ≥16 years hospitalized with pneumococcal bacteremia who died	Age ≥16 years hospitalized with pneumococcal bacteremia who survived	Univariable	Appropriate
Alanee et al. (2007)[21]	Multi-national , multicenter	Prospective Cohort	796	Age ≥15 years hospitalized with pneumococcal bacteremia (1) Patients infected with "invasive" serotypes (2) Patients infected with	Age ≥15 years hospitalized with pneumococcal bacteremia (1)Patients infected with all other serotypes (2) Patients infected with all other serotypes	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
				"pediatric" serotypes (3) Patients infected with PCV7 vaccine serotypes	(3) patients infected with non-PCV7 vaccine serotypes		
Aspa et al. (2006)[60]	Spain	Prospective cohort	638	Patients with pneumococcal CAP who died within 30 days	Patients with pneumococcal CAP who survived to 20 days	Univariable and Multivariable	Appropriate
Auburtin et al. (2001)[46]	France	Retrospective cohort	80	Pneumococcal meningitis patients ≥18 years old admitted to ICU who died	Pneumococcal meningitis patients ≥18 years old admitted to ICU who survived	Univariable and Multivariable	Appropriate
Baddour (2004)[57]	Multi-national	Prospective cohort	592	Patients ≥15 years with pneumococcal bacteremia who died within 14 days	Patients ≥15 years with pneumococcal bacteremia who	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
				of presentation	survived past 14 days		
Barsic et al. (1992)[45]	Croatia	Case-control	Cases: 21 Controls: 49	≥60 years hospitalized with pneumococcal meningitis	8-59 years hospitalized with pneumococcal meningitis	Univariable	Not appropriate
Berjohn et al. (2008)[143]	Philadelphia	Retrospective cohort	363 (38% of eligible patients)	Hospitalized patients ≥18 years old with pneumococcal bacteremia who died	Hospitalized patients ≥18 years old with pneumococcal bacteremia who survived	Univariable and Multivariable	Appropriate
Bruyn et al. (1989)[44]	Netherlands	Retrospective cohort	38	≥15 years of age hospitalized with pneumococcoccal meningitis who died	≥15 years of age hospitalized with pneumococcoccal meningitis who survived	Univariable	Appropriate
Calbo et al. (2009)[149]	Spain	Matched Case Control	Cases: 45	Patients with bacteremic pneumococcal	Patients with bacteremic pneumococcal	Univariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
			Controls: 90	pneumonia and COPD	pneumonia, no COPD, matched to cases by age(±5 years), gender, date positive culture		
Castillo et al. (2000)[150]	California	Retrospective cohort	281	Patients with bacteremia caused by penicillin non susceptible *S. pneumoniae*	Patients with bacteremia caused by penicillin susceptible *S. pneumoniae*	Univariable	Appropriate
Dwyer et al. (2006)[62]	Multi-national,	Prospective cohort	340	Patients with pneumococcal pneumonia who died	Patients with pneumococcal pneumonia who survived	Univariable and Multivariable	Appropriate
Eisen et al. (2006)[151]	Collected individual patient data from other studies, Multi-national,	Reanalysis-retrospective cohort	1642	Individual patient data from patients who died in studies that measured mannose-binding lectin levels	Individual patient data from patients who survived in studies that measured mannose-binding	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
	multicenter				lectin levels		
Feiken et al. (2000)[86]	Several states in USA and metropolitan Toronto/Peel, Ontario	Prospective cohort/ surveillance	12,194	All patients with IPD in the selected areas of surveillance who died	All patients with IPD in the selected areas of surveillance who survived	Univariable and Multivariable	Appropriate
Fernàndez Guerrero et al. (2003)[102]	Madrid, Spain	Retrospective cohort	327	Immunocompromised patients with pneumococcoccemia	Non-Immunocompromised patients with other chornic conditions and pneumococcoccemia	Univariable and Multivariable	Appropriate
Frankel et al. (1996)[101]	New Haven, Connecticut	Retrospective cohort	153	Patients with IPD who died	Patients with IPD who survived	Univariable	Appropriate
Garnacho-Montero (2010)[142]	Spain	Retrospective	125	Patients with IPD who died in hospital or within 90 days	Patients with IPD who survived	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
Grau et al. (2009)[112]	Barcelona, Spain	Prospective cohort	199	Adults ≥ 18 years with HIV infected with *S. pneumoniae* who died	Adults ≥ 18 years with HIV infected with *S. pneumoniae* who survived	Univariable and Multivariable	Appropriate
Harboe et al. (2009)[12]	Denmark	Retrospective cohort	18,858	Patients with IPD due to every serotype that occurred >50 times in sample	Patients with IPD due to serotype1	Univariable and Multivariable	Appropriate
Hsu et al. (2008)[152]	Singapore	Retrospective cohort	192	Patients with IPD in Singapore hospital who died	Patients with IPD in Singapore hospital who survived	Univariable	Appropriate
Imran et al. (2005)[153]	Singapore	Retrospective cohort	38	Patients with IPD who died	Patients with IPD who survived	Univariable	Appropriate
Kalin et al. (2000)[139]	Multinational	Prospective cohort	460	Patients with IPD who died	Patients with IPD who survived	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
Kim et al. (2002)[138]	Seoul, Korea	Retrospective cohort	199	Patients with IPD who died	Patients with IPD who survived	Univariable	Appropriate
Kirkpatrick et al. (1994)[43]	United Kingdom	Retrospective cohort	77 episodes in 69 patients	Patients with pneumococcocal meningitis who died	Patients with pneumococcocal meningitis who survived	Univariable	Appropriate
Kuikka et al. (1992)[154]	Helsinki	Retrospective cohort	159 episodes in 157 patients	Patients with IPD who died within 30 days of positive culture	Patients with IPD who survived	Univariable and Multivariable	Appropriate
Kumashi et al. (2005)[155]	Houston, Texas	Retrospective cohort	135 episodes in 122 patients	Patients with Cancer and IPD bloodstream infection who died	Patients with Cancer and IPD bloodstream infection who survived	Univariable and Multivariable	Appropriate
Lefort et al. (2000)[85]	France	Retrospective case series	30	Patients with pneumococcocal endocarditis who died	Patients with pneumcoccal endocarditis who survived	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
Lexau et al. (2005)[72]	USA counties	Prospective cohort	9934	Adults ≥ 50 years with IPD who died	Adults ≥ 50 years with IPD who survived	Univariable	Appropriate
Luján et al. (2010)[16]	Barcelona, Spain	Prospective cohort	299	Adults ≥18 with pneumonia and blood culture positive for *S. pneumoniae* who died	Adults ≥18 with pneumonia and blood culture positive for *S. pneumoniae* who died	Univariable and Multivariable	Appropriate
Martens et al. (2004)[14]	Copenhagen	Retrospective cohort	464	Adults ≥16 years with first episode of bacteremic pneumococcal disease who died	Adults ≥16 years with first episode of bacteremic pneumococcal disease who survived	Univariable and Multivariable	Appropriate
Maugein et al. (2002)[156]	France	Prospective surveillance	919	Patients with non-meningitis IPD who died	Patients with non-meningitis IPD who survived	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
McKenzie et al. (2000)[157]	Scotland	Prospective inception cohort	98	Patients with IPD who died	Patients with IPD who survived	Univariable	Appropriate
Moine et al. (1995)[158]	France, multicenter	Prospective cohort	43	Patients with severe pneumococcal CAP requiring ICU care who died	Patients with severe pneumococcal CAP requiring ICU care who survived	Univariable	Appropriate
Musher et al. (2000)[144] (Primary purpose to compare bacteremic and non-bacteremic pneumonia)	Houston, Texas	Prospective inception cohort	100	Patients with bacteremic and non-bacteremic pneumococcal pneumonia who died	Patients with bacteremic and non-bacteremic pneumococcal pneumonia who survived	Univariable	Appropriate
Nueman et al.	USA,	Case-control	1574	Patients with	Patients with	Univariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
(2006)[159]	multicenter			pneumococcal bacteremia who died	peumococcal bacteremia who survived	and Multivariable	
Pallares et al. (1987)[160]	Barcelona, Spain	Case Control	Cases: 24 Controls: 48	Adults with bacteremic pneumonia due to penicillin resistant pneumococcus	Adults with bacteremic pneumonia due to penicillin sensitive pneumococcus	Univariable	Appropriate
Redelings et al. (2005)[161]	California, USA	Retrospective cohort (death records)	5,265	Patients with characteristic of interest who died of IPD	Patients without characteristic of interest who died of IPD	Univariable	Appropriate
Rello et al. (2009)[162]	Spain	Prospective cohort	93	Adults hospitalized with pneumococcal CAP who died	Adults hospitalized with pneumococcal CAP who survived	Univariable and Multivariable	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
Song et al. (2004)[117]	9 Asian countries	Prospective cohort	233	Adults ≥18 years who presented with pneumococcal pneumonia and died	Adults ≥18 years who presented with pneumococcal pneumonia and survived	Univariable and Multivariable	Appropriate
Tleyjah et al. (2006)[119]	Multiple locations	Meta-analysis of prospective cohorts	10 studies; 3,430 patients	Patients with non-meningeal IPD due to PNSP who died.	Patients with non-meningeal IPD due to PSSP who died.	Univariable and Multivariable	Appropriate
Torres et al. (1998)[163]	Philadelphia	Retrospective cohort	71	Patients with IPD who died	Patients with IPD who survived	Descriptive only	Appropriate
Vallès et al. (2006)[137]	Barcelona, Spain	Prospective Cohort	125	Patients with pneumococcal CAP who died	Patients with pneumococcal CAP who survived	Univariable and Multivariable	Appropriate
Weinberger et al. (2010)[18]	Multiple locations	Meta-analysis	9 Studies,	Patients with pneumococcal pneumonia who	Patients with pneumococcal pneumoniae who	Random Effects (multivariable)	Appropriate

Author (Year)	Location	Study Design	N	Study Population	Comparison or Control Population	Multivariable or Univariable Analysis	Appropriateness of comparison population
				died	survived		
Weisholtz et al. (1983)[164]	New York	Retrospective cohort	264	Patients with pneumococcal bacteremia who died due to infection	Patients with pneumococcal bacteremia who survived	Univariable	Appropriate
Yu et al. (2003)[118]	Multinational	Prospective cohort	844	Adults ≥15 years with pneumococcal bacteremia who died within 14 days	Adults ≥15 years with pneumococcal bacteremia who survivedto 14 days	Univariable and Multivariable	Appropriate

A.2. Literature Search – Risk factors for Mortality due to IPD

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
Afessa et al. (1995)[114]	Chi squared comparing case fatality rates % per risk factor group	Age: < 65 years versus ≥ 65 years	31% vs 51%	0.025
		Mean arterial pressure: ≥ 65 mm Hg versus < 65 mm Hg	30% vs 73%	<0.0001
		Temperature (°F): ≥ 100 versus 97-99.9 versus < 97	25% vs 53% vs 83%	<0.001
		Respirations(/min): < 27 versus ≥ 27	27 vs 41	<0.0119
		WBC count (/mm^3): ≥ 10,000 versus 3,500-10,000 versus < 3,500	21% vs 47% vs 83%	<0.001
		Severity of disease: Mild versus Moderate versus Severe	13% vs 45% vs 79%	<0.001
		Platelets (/mm^3):	28% vs 39% vs 65%	<0.001

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		≥ 150,000 versus 100,000-150,000 versus < 100,000		
		Prothrombin time/control time ratio: < 1.2 versus ≥ 1.2	33% vs 73%	<0.001
		Albumin (g/dL): ≥ 3.5 versus 2-3.5 versus < 2	14% vs 37% vs 67%	<0.001
		Lactate dehydrogenase (U/mL): < 250 versus 250-400 versus ≥ 400	22% vs 31% vs 44%	0.0303
	Chi squared or Fisher exact % fatal vs % non fatal cases	Mechanical ventillation	86% vs 32%	<0.05
		Adult respiratory distress syndrome	36% vs 3%	<0.05
		Pulmonary embolism	3% vs 0%	<0.05 (Fisher)
		Seizure	16% vs 2%	<0.05 (Fisher)
		Acute renal dysfunction	57% vs 8%	<0.05
		Gastrointestinal bleeding	12% vs 3%	<0.05

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
Alanee et al. (2007) [21]	Multivariable logistic regression: OR (95% CI)	Serotype (More Invasive)	1.5 (0.7-3.4)	NS
		Age ≥65	2.5 (1.3-4.7)	0.004
	Exposure: More Invasive Serotypes (Serogroups 1, 5, 7)	Underlying chronic disease	2.0 (1.1-3.8)	0.025
		Immunosuppression	1.9 (1.1-3.4)	0.035
		Severity of illness (Pitt score >4)	16.1 (7.5-34.5)	<0.001
	Multivariable logistic regression: OR (95% CI)	Serotype (Pediatric)	1.2 (0.8-1.9)	NS
		Age ≥65	2.8 (1.6-4.7)	<0.001
	Exposure: Pediatric serotypes (Serogroups 6, 9, 19, 14, 23)	Underlying chronic disease	1.8 (1.1-3.0)	0.028
		Immunosupression	2.9 (1.8-4.7)	<0.001
		Severity of illness (Pitt score >4)	20.6 (12.5-34.0)	<0.001
		Nosocomial Infection	2.2 (1.1-4.9)	0.043
	Multivariable logistic regression: OR (95% CI)	Serotype (Conjugate)	0.84 (0.5-1.4)	NS
		Age ≥65	2.8 (1.6-4.9)	<0.001

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
	Exposure: Conjugate Vaccine (PCV7) Serotypes	Underlying chronic disease	1.8 (1.1-3.0)	0.025
		Immunosuppression	2.93 (1.8-4.8)	<0.001
		Severity of illness (Pitt score >4)	20.84 (12.6-34.3)	<0.001
		Nosocomial Infection	2.38 (1.1-5.2)	0.029
Aspa et al. (2006)[60]	Cox Regression Model HR (95% CI)	Bilateral disease	2.0 (1.2-3.1)	0.004
		Aspiration	2.8 (1.6-5.0)	0.001
		Shock	5.8 (3.4-9.8)	<0.001
		HIV infection	2.1 (1.1-3.8)	0.022
		Renal failure	1.9 (1.1-3.1)	0.019
		PSI score categories I-III vs. IV	2.6 (1.3-5.4)	0.010
		PSI score categories I-III vs. V	3.2 (1.5-6.9)	0.002
Auburtin et al.	Logitistic Regression	Platlet count <100G/L	32.7 (3.2-332.5)	0.0032

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
(2011) [46]	OR (95% CI)	Platlet count <100G/L	32.7 (3.2-332.5)	0.0032
		Arterial pH > 7.47	33.1 (3.4-319.7)	0.0025
		Mechanical ventillation	48.8 (2.6-901.5)	0.009
Baddour et al. (2004) [57]	Logistic Regression adjusted for HIV status OR (95% CI) Outcome = survival to 14 days	Combination antibiotic therapy	3.2 (1.1-9.2)	0.028
		HIV status	0.09 (0.02-0.3)	<0.001
	Logistic Regression adjusted for mechanical ventillation OR (95% CI) Outcome = survival to 14 days	Combination antibiotic therapy	2.9 (1.1-7.7)	0.04
		Mechanical ventillation	8.1 (3.0-2.2 (error?))	<0.001
Barsic et al. (1992)[45]	Not Included: inappropriate analysis comparing ages <60 to ≥60 years. Risk factors found may be accounted for by age.			

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
Berjohn et al. (2008) [143]	Logistic Regression adjusted for pneumonia severity index OR (95% CI)	Receipt of at least one antibiotic within 4 hours of presentation	0.4 (0.2-1.0)	0.04
		Receipt of at least one antibiotic within 8 hours of presentation	1.1 (0.4-3.2)	0.82**
Bruyn et al. (1989) [44]	Wilcoxon (continuous) Mean (SD) Chi Squared (discreet) %	Age	44 (18) vs 65 (17)	0.002
		Pulse Rate (/min)	97 (19) vs 115 (25)	<0.05
		ESR (mm/1st h)	36 (32) vs 84 (39)	<0.001
		Serum sodium level (mmol/l)	137 (4) vs 133 (6)	<0.05
		Serum bilirubin level (μmol/l)	15 (16) vs 56 (102)	<0.05
		Duration of illness before treatment: 8 to 17 days	4% vs 46%	<0.05
		Nuchal Rigidity (protective)	88% vs 38.5%	<0.01
		Presence of pneumonia with meningitis presentation	4% vs 38.5%	0.02
		Prior head trauma (protective)	56% vs 15.4%	<0.05

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
Calbo et al. (2009) [149]	Not included: Analysis for mortality risk factors was inappropriate given the matched case-control nature of the data.			
Castillo et al. (2000) [150]	Simple logistic regression, X^2	Penicillin non-susceptible *S. pneumoniae*	0.38 (0.09-1.65)**	0.19
Dwyer et al. (2006) [62]	Logistic Regression OR (95% CI)	Age >65 years	2.8 (1.2-5.9)	0.020
		≥2 lung lobes affected	2.2 (1.0-4.7)	0.045
		APS 8-14 vs APS 1-4	8.3 (2.1-54.8)	0.007
		APS 14-17 vs APS 1-4	23.8 (4.77-180.3)	0.0004
		APS ≥ 18	53.8 (11.8-395.0)	<0.0001
Eisen et al. (2008) [151]	Binary logistic regression	Higher median Age	1.09 (1.03-1.16)	<0.01
		Mannose binding lectin deficiency	5.62 (1.27 24.92)	0.02
Feiken et al. (2000) [86]	Logistic regression OR (95% CI)	Age 18-64 (≤17 as reference)	5.1 (1.2-21.0)	0.026
		Age 65-74 (≤17 as reference)	5.8 (1.4-25.0)	0.017

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Age ≥ 75 (≤17 as reference)	12 (2.8-49.0)	<0.001
		Asian race (Caucasian as reference)	1.9 (1.0-3.5)	0.042
		Living in Toronto/Peel Area (Connecticut as reference)	1.5 (1.0-2.3)	0.037
		Underlying disease present	2.8 (2.0-3.9)	<0.001
Fernàndez Guerrero et al. (2003) [102]	Cox-Mantel test (Multivariable)	Multilobar pneumonia	15.7 (6.0-41.3)	<0.001
		Inappropriate therapy	12.2 (4.1-37.0)	<0.001
		Obtundation	5.8 (2.2-15.0)	<0.001
		Hospital-acquired bacteremia	4.8 (1.0-14.6)	<0.006
Frankel et al. (1996) [101]	Test of proportions	Elderly (>70 years)	29% vs. 10%	<0.01
		HIV Positive	13% vs. 12%	>0.05**
Garnacho-Montero (2010) [142]	Cox Proportional Hazards Model, outcome=in-hospital mortality Adjusted HR (95% CI)	Delay >4 hrs from admission to start of adequate antibiotic treatment	2.6 (1.06–6.5)	0.037
		Sepsis or Septic Shock	5.06 (1.63–15.71)	0.005

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
	Cox Proportional Hazards Model, outcome=90 day mortality Adjusted HR (95% CI)	Charlson comorbidity index	1.2 (1.02–1.34)	0.018
		Severe sepsis or septic shock	3.03 (1.2–7.5)	0.016
		Delay >4 hrs from admission to start of adequate antibiotic treatment	2.21 (1.0–4.9)	0.048
Grau et al. (2009) [112]	Logistic regression	Living in the Late HAART era	NR	0.017
		Shock at presentation	7.0 (2.1-23.9)	0.002
		Associated comorbidities	4.3 (1.5-11.9)	0.006
Harboe et al. (2009) [12]	Logistic regression (adjusted) OR (95% CI) Each serotype compared in separate regression to reference serotype (serotype 1)	In patients ≥5 years: Infection with serotypes 31, 11A, 35F, 17F, 3, 16F, 19F, 15B, 10A (relative to serotype 1)	OR ≥3	<0.05
		In patients ≥5 years: Infection with serotypes 19A, 23A, 9N, 6B, 23F, 6A, 18C, 24F, 14, 12F, 20, 22F, 9V, 4, 8, 5, 38 (relative to serotype 1)	OR's between 1.55 and 2.91	<0.05
		For patients <5 years	No serotypes statistically significant due to low	NS

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
			numbers of deaths in children	NS
		Age per 1 year increment	1.02 (1.02-1.03)	<0.001
		Male	1.19 (1.09-1.29)	<0.001
		Meningitis diagnosis	1.9 (1.7-2.14)	<0.001
		High Charlson index (versus low index)	1.85 (1.66-2.07)	<0.001
		Intermediate Charlson index (versus low index)	1.35 (1.23-1.48)	<0.001
		History of alcoholism related conditions	2.40 (2.08-2.78)	<0.001
		Earlier decade of diagnosis: 1977-1986 compared to 1997-2007	1.33 (1.16-1.52)	<0.001
		Earlier decade of diagnosis: 1987-1996 compared to 1997-2007	1.20 (1.10-1.31)	<0.001
Hsu et al. (2008) [152]	Crude Logistic Regression OR (95% CI)	Fulfillment of the Advisory Committee on Immunization Practices criteria for PPV vaccination	8.1 (1.9-35.2)	0.005
Imran et al.	Tests of proportions	Presence of septic shock on admission	6	<0.005

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
(2005) [153]	N	Presence of septic shock on admission	6	<0.005
		Underlying Malignancy	6	0.008
		Leukopenia or leukocytosis	3	<0.005
		Haemoglobin level <12g/dL (anemia)	19	0.021
		Presence of High anion gap	15	0.047
Kalin et al. (2000) [139]	Logistic Regression OR (95% CI)	Aged >65 years	2.2 (1.1-4.4)	0.026
		Residence in nursing home	2.8 (1.0-7.3)	0.043
		Chronic pulmonary disease	2.5 (1.2-5.1)	0.014
		Acute physiology score (APS) 9-14 compared to <9	7.6 (2.4-33.0)	0.002
		APS 15-17 compared to <9	22 (5.8-112)	<0.0001
		APS ≥ 18 compared to <9	41 (12.0-194.0)	<0.0001

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
Kim et al. (2002) [138]	Tests of proportions No. deaths/No. cases (%)	Age ≥ 65 years	18/50 (36.0)	0.01
		Neurologic disease	3/ 4 (75.0)	0.04
		Atineoplastic chemotherapy	9/18 (50.0)	0.01
		Indwelling urinary catheter	3/3 100.0	0.01
		Bedridden state	3/3 (100.0)	0.01
		Leukopenia	16/33 (48.5)	<0.001
		Polymicrobial bacteremia	9/14 (64.3)	0.001
		Septic Shock	19/27 (70.4)	<0.001
		Respiratory failure	14/19 (73.7)	<0.001
		Deteriorated mental status	18/37 (48.6)	<0.001
		Acute renal failure	11/13 (84.6)	<0.001
		Disseminated Intravascular coagulation	3/ 4 (75.0)	0.04
		ICU admission	14/37 (37.8)	0.02

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Seizure	3/ 4 (75.0)	0.04
Kirkpatrick et al. (1994) [43]	Chi Squared N (%)	Age > 60 years	4 (33.3)	<0.05
		Presence of a chest infection	N and % not reported	<0.05
		Presence of acidosis	N and % not reported	<0.05
		CSF protein >10 g/l	N and % not reported	<0.05
Kuikka et al. (1992) [154]	Logistic Regression OR (95% CI)	Thrombocytopenia (platelets <100 x 10^9 /l) at time of positive culture	21.5 (2.6-176.9)	0.004
		Circulatory acidosis (Circulatory pH < 7.35) at time of positive culture	15.2 (2.0-113.0)	0.008
Kumashi et al. (2005) [155]	Univariable Fisher Exact stratified by Penicillin Nonsusceptibility	No significant risk factors	_	_
Lefort et al. (2000) [85]	Chi Square tests with Yates correction where appropriate. Checked with	Age ≥ 65 years	3.1	<0.05
		Presence of septic shock	3.4	<0.05

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
	logistic regression RR (no 95% CI reported)	Cardiac surgery (protective)	0.43	<0.05
Lexau et al. (2005) [72]	Logistic Regression (Univariable)	Age ≥ 75-84 years	1.5 (1.2-1.8)	NR
		Age ≥ 85 years	2.7 (2.2-3.3)	NR
		Meningitis	1.7 (1.3-2.3)	NR
		Bacteremia without focus	1.6 (1.3-1.9)	NR
		One or more immunocompromising conditions	1.5 (1.3-1.8)	NR
		Two or more chronic conditions	1.5 (1.3-1.8)	NR
		Infected with serotype 19F (reference group = serotype 14)	2.1 (1.4-3.1)	NR
		Infected with serotype 23F	1.5 (1.0-2.1)	NR
		Infected with serotype 3	2.1 (1.5-2.8)	NR

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Infected with serotype 11A	2.1 (1.4-3.2)	NR
Luján et al. (2010) [16]	Logistic Regression with ATS/IDSA criteria OR (95% CI)	Low invasive potential/opportunistic serotypes (high invasive=baseline)	7.0 (1.7-28.6)	<0.01
		Alcohol abuse	4.0 (1.4-11.4)	0.01
		ATS/IDSA criteria	4.8 (1.9-12.1)	<0.01
		Higher mean Charlson score	1.3 (1.1-1.6)	<0.01
		Higher mean age	1.02 (0.99-1.05)	0.11**
	Logistic Regression with PSI OR (95% CI)	Low invasive potential/opportunistic serotypes (high invasive=baseline)	5.3 (1.3-21.6)	<0.05
		PSI class V	9.5 (3.1-29.5)	<0.001
		Alcohol Abuse	3.2 (1.2-8.5)	<0.05
		Higher mean Charlson score	1.2 (1.0-1.5)	<0.05
Martens et al.	Cox proportional hazard	Serotype 3	2.5 (1.2-5.3)	NR

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
(2004) [14]	regression RR (95% CI)	Serotype 3	2.5 (1.2-5.3)	NR
		Serotype 1 (protective)	0.23 (0.06-0.97)	NR
		Age 50-64 (reference group = 16-49 years)	2.23 (1.0-5.0)**	NR
		Age 65-79	3.2 (1.5-6.8)	NR
		Age >80	2.9 (1.2-6.7)	NR
		Comorbidity	1.2 (0.8-2.0)**	NR
		Alcoholism	1.8 (1.0-3.42)**	NR
		B leukocyte count $\leq 9x10^9$/L	2.8 (1.7-4.6)	NR
		Temperature < 38.5°C	1.7 (1.1-2.6)	NR
Maugein et al. (2003) [156]	Logistic Regression OR (95% CI)	Age >60 years	5.3 (1.5-18.5)	0.01
		Immunodeficiency	1.4 (1.0-1.9)**	0.05
McKenzie et	Case fatality rates reported,	Age	43%	None

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
al. (2000) [157]	no tests of significance *authors note sample size too small to claim significance with mortality.	Age	43%	None
		Serotype 6A infection 3 of 4 cases died.	75%	None
		Serotype 19A infection 3 of 5 cases died	60%	None
Moine et al. (1995) [158]	Wilcoxon test for continuous variables	Higher Mean SAPS Deaths vs survivors	18.1 ± 5.3 vs. 11.7 ± 2.9	0.0001
		Septic shock at onset of disease	NR	0.001
	Logrank tests	Presence of impaired alertness	NR	0.026
		Mechanical ventilation required on admission	NR	0.0009
		Mechanical ventilation required during hospitalization	NR	0.003

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Acute renal failure	NR	0.01
		Bacteremic pneumonia	NR	≤0.05
Musher et al. (2000) [144]	Univariable Logistic Regression	Bacteremic pneumonia associated with death within 7 days (compared to non-bacteremic pneumonia)	OR: 5.58 (No CI reported)	0.02
		High PORT score together with bacteremic pneumonia Case fatality rate in bacteremic versus not bacteremic with high PORT score	Case fatality rate 30% versus 10%	NR
Nueman et al. (2006) [159]	Multivariable Logistic Regression OR (95% CI)	Any antibiotic non-susceptible strain (including macrolide, penicillin, cephalosporin)	1.4 (1.02-2.1)	NR
		Inpatient or admitted after blood culture taken in Emergency or clinic	5.1 (2.0-13.0)	NR
		Age (per 10 year increase)	1.3 (1.2-1.4)	NR
		Male	1.4 (1.02-2.0)	NR
		Black Race (compared to White)	0.6 (0.4-0.9)	NR

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Focal Infection	0.6 (0.4-0.9)	NR
Pallares et al. (1987) [160]	Fisher's exact test	Infection with penicillin resistant pneumococcus	54% vs 25%	0.03
Redelings et al. (2005) [161]	Linear Regression	Age	8.5% increase in risk per year increase in Age	NR
	Crude Mortality Rates Rate Ratio (95% CI)	Males with pneumonia compared to females with pneumonia, adjusted for age	1.68 (1.58-1.78)	NR
		Males with septicemia compared to females with septicemia, adjusted for age	1.56 (1.25-1.94)	NR
		African American, adjusted for age	1.38 (1.25-1.53)	NR
Rello et al. (2009) [162]		Bacterial load >1,000 copies/mL	5.43 (1.52-19.24)	NR
Song et al. (2004) [117]	Logistic regression OR (95% CI)	Bacteremia	10.5 (2.9-37.8)	<0.01
		Mechanical ventilation	12.5 (3.6-42.9)	<0.01
		Antibiotic non-susceptibility	1.5 (0.4-5.3)	0.4**

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Discordant antibiotic therapy	0.9 (0.3-2.6)	0.9**
Tleyjah et al. (2006) [119]	Meta-analysis of adjusted OR and corresponding RR (95% CI)	Penicillin Non-susceptible Infections	Adjusted OR: 1.37 (1.05-1.78) Adjusted RR: 1.29 (1.04-1.59)	
Torres et al. (1998) [163]	Descriptive only, reported proportions. Percent with risk factor vs percent without	Leukopenia	50% vs 24%	NR
		Lack of fever	64% vs 19%	NR
		Age >70 years	73% vs 20%	NR
Vallès et al. (2006) [137]	Stepwise Logistic Regression RR* (95% CI) Note: study said used Logistic regression but reports Relative risk.	Female gender	9.1 (1.3-61.2)	0.02
		Oral corticoid steroid therapy prior to hospitalization	10.6 (1.2-92.2)	0.03
		Pleural effusion	13.4 (1.9-93.1)	0.009
		Comorbidity	1.9 (0.3-13.9)	0.5**

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		Penicillin Non-suscetibility	4.2 (0.7-25.2)	0.1**
Weinberger et al. (2010) [18]	Random Effects model of combined RR from meta-analysis RR (95% CI) compared to serotype 14	Pneumococcal pneumonia infection due to serotype 3, 6A, 6B, 9N, 19F	Graph only, numbers not reported. These 5 serotypes reported as significantly associated with increased risk of mortality	NR
Weisholt et al. (1983) [164]	Chi-squared tests Proportion death in patients with risk factor versus proportion in comparison group	15-59 years olds versus 2-14 year olds	18% versus 6 %	<0.05
		≥60 year olds versus 2-14 year olds	27% versus 6%	<0.001
		≥60 year olds versus 15-59 year olds	27% versus 18%	<0.1
		Disease considered to be associated with significant impairment of host immunity (see paper for breakdown) versus patients considered to be normal healthy host	23% versus 8%	<0.01
Yu et al. (2003) [118]	Logistic Regression OR (95% CI)	Age >65 years old	2.9 (1.6-5.2)	0.0004
		Critical Illness (Pitt bacteremia score	21.1 (12.5-35.6)	0.0001

Author (Year)	Type of analysis	Mortality Risk Factors	Results of analysis	P-value
		>4)		
		Underlying disease or risk factor associated with immunosuppression	3.1 (1.8-5.3)	0.0001
		Underlying chronic disease	1.3 (0.7-2.2)	> 0.2**
		Penicillin susceptibility in vitro	1.4 (0.8-2.4)	0.19**

** Not significant; NR=None reported, see 95% CI for significance

APPENDIX B: ATMOSPHERIC REQUIREMENTS FOR GROWTH ON MEDIUMS

Agar Type	Temperature	Atmospheric Requirements
Blood Agar	35ºC	Carbon Dioxide
Chocolate Agar	35ºC	Carbon Dioxide
Colistin Nalidixic Acid Blood Agar (CNA)	35ºC	Carbon Dioxide
MacConkey with Crystal Violet Agar	35ºC	Oxygen
Brucella Agar	35ºC	Anaerobic Conditions^
Kanamycin Vancomycin Laked Blood Agar (KV)	35ºC	Anaerobic Conditions^
Anaerobic Phenyl Ethyl Alcohol Agar (PEA)	35ºC	Anaerobic Conditions^
Bile Esculin Azide Agar (BEAA)	35ºC	Anaerobic Conditions^

^Anaerobe jars are used with the Anoxomat Gas System to replace the air in the jars with anaerobic conditions.

APPENDIX C: CREATING MODIFIED CHARLSON COMORBIDITY INDEX

C.1. Matching CASPER Data to Charlson Comorbidity Index Weighting for Modified Charlson Index

Charlson Weighting	Carlson Comorbidity List	CASPER Comorbidity
1	Myocardial Infarction	Myocardial Infarction
	Congestive Heart Failure	Congestive heart failure
	Peripheral vascular disease	Peripheral vascular disease
	Cerebrovascular disease	Stroke
	Dementia	Alzheimer's or other dementia
	Chronic Pulmonary Disease	Chronic obstructive pulmonary disease
		Asthma
		Chronic bronchitis
		Emphysema
		Pulmonary fibrosis

Charlson Weighting	Carlson Comorbidity List	CASPER Comorbidity
		Restrictive lung disease
		Cystic lung disease
		Pulmonary hypertension
		Bronchiectasis
		Systemic lupus erythematosus
	Connective Tissue disease	Polymyositis
		Rheumatoid arthritis
		Autoimmune connective tissue disorder
1	Ulcer Disease	Peptic ulcer disease
		Alcoholic hepatitis
		Hepatitis A without cirrhosis
	Mild Liver Disease	Hepatitis B without cirrhosis
		Hepatitis C without cirrhosis

Charlson Weighting	Carlson Comorbidity List	CASPER Comorbidity
		Other hepatitis
		TPN Cholestasis
		Cholecystitis
		Stenosis of liver
	Diabetes	Diabetes
	Hemiplegia	Hemiplegia
		Chronic renal failure
		Pre-renal failure
		Nephrolithiases
2	Moderate/severe renal disease	End stage renal failure
		Chronic renal insufficiency
		Dialysis patient
		Nephritis

Charlson Weighting	Carlson Comorbidity List	CASPER Comorbidity
	Diabetes with end organ damage	Unable to match
	Any tumour	See Appendix C.2
	Leukemia	See Appendix C.2
	Lymphoma	See Appendix C.2
3	Moderate or severe liver disease	Hepatic cirrhosis Hepatic/Liver failure Biliary cirrhosis Portal Hypertension Esophageal Varicies (Sequela of severe liver disease) Gastric Varicies (Sequela of severe liver disease)
6	Metastatic solid tumour	See Appendix C.2
	AIDS	HIV/AIDS

C.2. Cancer Types as Collected in CASPER Data Classified into Charlson Index Cancer Classifications

Cancer in CASPER database	Charlson Index Classification
Adenocarcinoma of esophagus	Any tumour
Adenocarcinoma of duodenum (metastasized)	Metastatic solid tumour
Adenocarcinoma of lung	Any tumour
Adenoma	Any tumour
AML	Leukemia
B-cell lymphoma	Lymphoma
Basal cell carcinoma	Any tumour
Bone marrow sarcoma	Any tumour
Bowel	Any tumour
Bowel - metastatic	Metastatic solid tumour
Breast cancer	Any tumour

Cancer in CASPER database	Charlson Index Classification
Breast cancer with metastases	Metastatic solid tumour
Cervical cancer	Any tumour
Chronic lymphocytic leukemia	Leukemia
CNS lymphoma	Lymphoma
Colon cancer	Any tumour
Colon cancer with metastases	Metastatic solid tumour
Endometrial	Any tumour
Esophageal	Any tumour
Follicular lymphadema (in remission)	Any tumour
Hurthle cell tumour, benign pleomorphism	Any tumour
Kidney Cancer	Any tumour
Leukemia	Leukemia
Lung	Any tumour

Cancer in CASPER database	Charlson Index Classification
Lung (end stage)	Any tumour
Lung / kidney (multiple myeloma)	Any tumour
Lung & retroperitoneal mass-hepatoid	Any tumour
Lung cancer non-small cell carcinoma	Any tumour
Lung with metastasis	Metastatic solid tumour
Liver lymphoma	Lymphoma
Follicular lymphoma	Lymphoma
Hodgkin's lymphoma	Lymphoma
Non-Hodgkin's lymphoma	Lymphoma
Lymphoma	Lymphoma
Lymphoma (mantle cell carcinoma)	Lymphoma
Lymphoma (to liver)	Lymphoma
Malignant lymphoma/diffuse large B-cell	Lymphoma

Cancer in CASPER database	Charlson Index Classification
Mantle cell lymphoma (non-Hodgkin's lymphoma)	Lymphoma
Metastatic breast carcinoma	Metastatic solid tumour
Metastatic cancer	Metastatic solid tumour
Metastatic pancreatic	Metastatic solid tumour
Multiple myeloma	Any tumour
Myeloma	Any tumour
Nasopharyngeal cancer	Any tumour
Non-small lung cancer, unresectable	Any tumour
Ovarian stage III	Metastatic solid tumour
Pancreatic cancer	Any tumour
Prostate cancer	Any tumour
Prostate with metastasis	Metastatic solid tumour
Remote basal cell carcinoma	Any tumour

Cancer in CASPER database	Charlson Index Classification
Renal cell carcinoma	Any tumour
RLL lesions - stage IV lung cancer	Metastatic solid tumour
Sarcoma with lower extremity metastasis	Metastatic solid tumour
Small cell lung carcinoma	Any tumour
Soft palate squamous cell	Any tumour
Squamous cell	Any tumour
Squamous cell cancer (throat), meds to	Metastatic solid tumour
Squamous cell carcinoma, right lung	Any tumour
Squamous metaplastic cells with atypia	Any tumour
Squamous cell cancer of lung	Any tumour
Stage IV breast cancer with bone and hepatic metastases	Metastatic solid tumour
Stage IV metastatic lung carcinoma	Metastatic solid tumour

Cancer in CASPER database	Charlson Index Classification
Synoid sheath sarcoma	Any tumour
T-cell lymphoma nasopharynx	Lymphoma
Throat, subglotic	Any tumour
Uterine	Any tumour
Uterine cancer and chronic lymphocytic leukemia	Leukemia

APPENDIX D: APPROPRIATE ANTIBIOTICS AND ROUTES OF ADMINISTRATION FOR TREATMENT OF IPD MENINGITIS OR NON-MENINGITIS MANIFESTATION

Key:
0 – Antibiotic not appropriate for treatment of IPD manifestation
1 – Antibiotic appropriate for treatment of this manifestation of IPD assuming pneumococcal sensitivity to antibiotic
2 – Antibiotic only appropriate for use as second drug in dual treatment of IPD

Antimicrobial Class	Antimicrobial Family	Antimicrobial Agent	Route of Administration	Meningitis	Non-Meningitis
Beta-lactam	Penicillin	Amoxicillin	PO	0	0
Beta-lactam	Penicillin	Amoxicillin/Clavulana	PO	0	0
Beta-lactam	Penicillin	Ampicillin	IV	1	1
Beta-lactam	Penicillin		PO	0	0
Beta-lactam	Penicillin	Cloxacillin	IV	1	1
Beta-lactam	Penicillin		PO	0	0
Beta-lactam	Penicillin	Penicillin	IV	1	1

Antimicrobial Class	Antimicrobial Family	Antimicrobial Agent	Route of Administration	Meningitis	Non-Meningitis
Beta-lactam	Penicillin		PO	0	0
Beta-lactam	Penicillin	Piperacillin	IV	0	1
Beta-lactam	Penicillin	Piptaz (Tazocin)	IV	0	1
Beta-lactam	1st generation cephalosporin	Cefazolin	IV	0	1
Beta-lactam	1st generation cephalosporin	Cephalexin	PO	0	0
Beta-lactam	2nd generation cephalosporin	Cefaclor	PO	0	0
Beta-lactam	2nd generation cephalosporin	Cefprozil	PO	0	0
Beta-lactam	2nd generation cephalosporin	Cefuroxime	IV	0	1
Beta-lactam	2nd generation cephalosporin		PO	0	0

Antimicrobial Class	Antimicrobial Family	Antimicrobial Agent	Route of Administration	Meningitis	Non-Meningitis
Beta-lactam	3rd generation cephalosporin	Cefotaxime	IV	1	1
Beta-lactam	3rd generation cephalosporin	Ceftazidime	IV	1	1
Beta-lactam	3rd generation cephalosporin	Ceftriaxone	IV	1	1
Beta-lactam	Carbapenem	Meropenem	IV	1	1
Macrolide	Macrolide	Azithromycin	IV	0	2
Macrolide	Macrolide		PO	0	2
Macrolide	Macrolide	Clarithromycin	PO	0	2
Macrolide	Macrolide	Erythromycin	IV	0	2
Macrolide	Macrolide		PO	0	2
Quinolone	Quinolone	Ciprofloxacin	IV	0	0

Antimicrobial Class	Antimicrobial Family	Antimicrobial Agent	Route of Administration	Meningitis	Non-Meningitis
Quinolone	Quinolone		PO	0	0
Quinolone	Quinolone	Gatifloxacin	IV	1	1
Quinolone	Quinolone		PO	0	1
Quinolone	Quinolone	Levofloxacin	IV	1	1
Quinolone	Quinolone		PO	0	1
Lincosamide	Lincosamide	Clindamycin	IV	2	1
Lincosamide	Lincosamide		PO	0	2
Aminoglycoside	Aminoglycoside	Gentamicin	IV	0	0
Aminoglycoside	Aminoglycoside	Tobramycin	IV	0	0
Aminoglycoside	Aminoglycoside	Amikacin	IV	0	0
Tetracycline	Tetracycline	Tetracycline	PO	0	0
Sulfonamide	Sulfonamide	TMP/SMX	IV	0	1

Antimicrobial Class	Antimicrobial Family	Antimicrobial Agent	Route of Administration	Meningitis	Non-Meningitis
Sulfonamide	Sulfonamide		PO	0	0
Nitroimidazole	Nitroimidazole	Metronidazole	IV	0	0
Nitroimidazole	Nitroimidazole		PO	0	0
Oxazolidinone	Oxazolidinone	Linezolid	IV	0	1
Oxazolidinone	Oxazolidinone		PO	0	1
Glycopeptide	Glycopeptide	Vancomycin	IV	1	1