The Role of *Streptococcus pneumoniae* in Community-Acquired Pneumonia

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Abstract

Streptococcus pneumoniae (the pneumococcus) remains one of the most common causes of bacterial CAP, encompassing infections mild enough to be treated on an outpatient basis, as well as those requiring hospital care, or even intensive care unit admission. This microorganism is associated with a significant burden of disease, causing substantial morbidity and mortality worldwide, and generating considerable health-care costs. The reason that pneumococcal CAP remains such a common cause of disease relates to the presence of a number of risk factors for this infection in patients throughout the world. Such risk factors include extremes of age, lifestyle factors, including smoking and alcohol abuse, and various underlying co-morbid conditions, including congenital and acquired immunodeficiencies. This article will review various aspects of pneumococcal CAP, including the burden of pneumococcal disease, risk factors for pneumococcal infection, the occurrence of cardiovascular events in patients with pneumococcal CAP, the apparently pivotal role of pneumolysin, a major virulence factor of the pneumococcus, in the pathogenesis of severe infection and associated cardiac dysfunction, empiric antibiotic treatment for pneumococcal CAP, as well as adjunctive therapies, specifically those which target pneumolysin, and, finally, the mortality of such infections.

Keywords

antibiotics - adjuvant therapy - biomarkers - burden of disease - cardiac events - community-acquired pneumonia - mortality - pneumococcus - pneumolysin - Streptococcus pneumoniae

Introduction

Community-acquired pneumonia (CAP) is associated with a significant clinical and economic burden of disease encompassing both developed and developing nations and is a cause of considerable morbidity and mortality [1–5]. In fact, the Global Burden of Disease Study 2010 documented that in that year, lower respiratory tract infections, which included CAP, were among the leading causes of death and of years of life lost (YLL) due to premature mortality [6]. When reviewing the various studies documenting the burden of CAP it becomes apparent that although there may be regional differences in the microbial etiologies of CAP, *Streptococcus pneumoniae* (the pneumococcus) is always noted to be the most commonly encountered pathogen [1–5]. It has, therefore, been said that in the consideration of any aspect of CAP, such as risk factors, antimicrobial resistance and antibiotic treatment, attention should always be paid to these factors in relationship to the pneumococcus, since the clinical characteristics of CAP are to a large extent dominated by the epidemiology of this pathogen. This current article will review various aspects of pneumococcal CAP, including details of the burden of disease, risk factors for infection, cardiac consequences, the seemingly pivotal role of pneumolysin in the pathogenesis of disease, antimicrobial treatment and adjunctive therapies, especially those which target pneumolysin.

Burden of pneumococcal disease

Much has been written about the ongoing burden of pneumococcal infections in the world [7–12]. In the first instance, it is important to recognize that pneumococcal infection can be classified as being either invasive or non-invasive [9–11]. The former includes bacteremic pneumonia and meningitis, while the latter, at least in

adults, consists predominantly of non-bacteremic pneumonia, which is by far the major burden of pneumococcal disease in adults [11]. It is clear that our understanding of the incidence of pneumococcal pneumonia comes mainly from studies of patients with bacteremic infections, which significantly underestimate the true burden of infection. One systematic review of the literature analysing the diagnostic yield of various microbiological tests used in the diagnosis of pneumococcal pneumonia estimated that for every case of bacteremic pneumococcal pneumonia there were an additional three cases of non-bacteremic disease [12].

As described above, a review of multiple studies has indicated that the pneumococcus remains the most common cause of CAP, irrespective of whether the infection is mild enough to be treated at home, or requires hospitalization, or even intensive care unit (ICU) admission [1–5, 13]. The review of the burden of CAP in adults in Europe (46 articles) confirmed that the pneumococcus was the most frequent pathogen accounting for 38% of CAP cases treated as outpatients, 27% of hospitalized cases and 28% of ICU cases [5].

It is said that pneumococcal pneumonia is associated with bacteremia in 10-30% of cases, constituting one of the more common invasive pneumococcal infections [9]. Rates of invasive pneumococcal disease (IPD) reported in European and US studies (undertaken before widespread use of pneumococcal conjugate vaccines in children) varied between 11 and 23.2 per 100,000 population, being even higher in the elderly and those with underlying comorbid conditions (for example 16.2 – 59.7/100,000 population in those > 65years) [9]. Other authors reviewing additional literature have

reported an incidence of IPD ranging from 11-27 per 100000 population in Europe and 15-49 per 100000 population in the US [11]. It is important to note that some studies have documented possible increases in the incidence of IPD over recent years [8, 11]. For example, while decreases in mortality due to IPD have been documented over the years, particularly in developed countries such as France, which have been attributed to improved socioeconomic conditions and the use of antibiotics and vaccination strategies, since 1993 there have been increases in the occurrence of IPD in all age groups other than children <2 years of age [8]. Part of this increase in pneumococcal disease has been said to be due to serotype fluctuations and/or vaccine serotype replacement disease, while many studies have also clearly documented the existence in those populations of large numbers of patients with one or more underlying, predisposing conditions as described more fully below [8, 11]. In fact, studies have suggested that IPD rates of 176-483/100,000 population occur in patients with chronic medical conditions and rates of 342-2031/100,000 population in patients with immunosuppression [9]. Very interestingly, a recent study from South Africa documented in HIV-infected adults that despite a stable prevalence of HIV infection and an increased roll-out of antiretroviral therapy, the burden of IPD had not decreased [7].

An additional aspect to consider with regard to CAP, in general, and pneumococcal infections, in particular, is the regular reporting of increasing levels of antibiotic resistance among the common pathogens worldwide [1, 3–5, 9, 14–16]. A detailed discussion of the problem of antimicrobial resistance is beyond the brief of the current manuscript, but has been reviewed elsewhere [14, 16]); however, it is important to recognize that while there is a concern that antibiotic resistance may

make treatment of CAP more difficult, and potentially be associated with poorer outcomes, many studies and reviews have indicated that antibiotic resistance cannot consistently be shown to have a negative impact on patient outcomes [1, 4, 5, 9, 14–16].

Mortality rates for IPD in the Western world range between 11-30%, and while decreases in IPD mortality have occurred following the introduction of the pneumococcal conjugate vaccine in countries such as the USA, some studies from Europe have documented no changes in IPD mortality [11]. In fact it has been suggested that the case fatality rate for hospitalized patients with IPD has not changed substantially since as early as 1952, and still currently remains at ~12% [10]. Furthermore, several studies clearly document that CAP is associated with considerable healthcare costs in many parts of the world [1, 4, 5, 9].

Risk factors for pneumococcal CAP/IPD

A number of studies have been undertaken in patients with CAP documenting risk factors for infection and have indicated that demographic features, lifestyle factors and underlying comorbid conditions are important contributors [13, 17]. Several similar studies have also documented the existence of these risk factors in patients with pneumococcal CAP, and particularly those with IPD [18–29]. Among the major risk factors for pneumococcal infection and/or IPD are extremes of age, lifestyle issues such as cigarette smoking and alcohol abuse, and various underlying comorbid conditions, as well as various congenital and acquired immunodeficiencies [20] (Table 1). With regard to smoking, one early study documented cigarette smoking to be the most significant independent risk factor for IPD, even among non-

TABLE 1. Risk factors for Invasive Pneumococcal Infections

Age
< 2 or ≥ 65 y
Ethnic groups
African descent
Alaskan natives
American Indians
Underlying clinical pulmonary diseases
Chronic obstructive pulmonary disease
Asthma
Other chronic clinical conditions
Chronic liver disease
Chronic renal failure
Nephrotic syndrome
Diabetes mellitus
Functional or anatomic asplenia
Sickle cell disease
Splenectomy
Substance abuse
Alcohol abuse
Smoking habit
Crack use
Cocaine use
Immunosuppressive conditions
HIV infection
Congenital immunodeficiency
Malignancy
B-cell defects
Multiple myeloma
Patients undergoing treatment
Alkylating agents
Antimetabolites
Systemic glucocorticoids
Patients with cerebrospinal fluid leaks
Cochlear implant recipients
Solid organ or hematopoietic cell transplant recipients
Patients with influenza

Source: Reproduced with permission from Wolters Kluwer (Aspa and Rajas).[25]

elderly, non-immunocompromised patients [18]. A more recent study documented that current smokers with pneumococcal CAP often developed severe sepsis, are younger at hospitalization, despite fewer comorbidities, and that smoking was an independent risk factor for 30-day mortality [26]. Alcohol use disorders have also

been shown to be associated with increased hospital mortality, length of hospital stay and costs in patients with pneumococcal CAP [28].

Comorbid conditions that are frequently documented risk factors for IPD include diabetes mellitus, chronic lung conditions, including asthma and COPD, and chronic heart disease, with conditions such as solid cancers, HIV/AIDS and hematological cancers having the greatest risk [19]. In a study of risk factors for IPD in England, chronic kidney and liver disease were important issues [22]. In the study by Kyaw and colleagues, risk ratios for IPD were 3 to 6-fold higher for patients with diabetes mellitus and chronic heart and lung disease, 11-fold higher in patients who abused alcohol, and 23 to 48-fold higher in patients with HIV/AIDS or cancers [19]. Risk ratios also increased progressively in those cases having more than one risk factor and with increasing age in association with one or more comorbid conditions. In that study, the risk for IPD was greater in Black adults compared with White adults, both in the healthy population and in those with comorbidity. In addition, other regional and ethnic issues have also been found to be important, with the risk of IPD being greater in various indigenous peoples of different areas of the world [20]. The risk of IPD is also increased in persons exposed to crowded conditions such as long-term care facilities, schools, day-care centres, prisons etc. [20]. The risk of IPD is also increased in patients with primary or acquired immunodeficiencies, including sickle cell disease, splenectomy or asplenia, HIV infection, and organ transplant recipients [20, 21, 29].

Interestingly, but very importantly, it is well recognized that there is an association and interaction between viral infections and bacterial respiratory tract pathogens with

the mechanisms of this interaction and their roles in the pathogenesis of CAP having been well elucidated and described [30–33]. There appears to be a particularly close association between viral infections, and in particular influenza infections, including both seasonal and pandemic influenza, with subsequent development of *S. pneumoniae* infections, the occurrence and mechanisms of which have also been well documented in both experimental animal and human studies [34–41]. While a detailed description of the pathogenic mechanisms is beyond the brief of this current manuscript, these have been described in many reviews [36, 37, 39–41]. Furthermore, influenza virus infections have been shown to be associated with elevated pneumococcal loads in the blood as assessed by PCR techniques, and are associated with an increased risk of death [42].

The important interactions between the influenza virus and the pneumococcus highlight the potential importance of the use of pneumococcal vaccination, particularly together with influenza vaccination. This strategy has variously been shown in the different studies to be effective, or is likely to be effective, in reducing influenza morbidity and associated pneumonia, while preventing pneumonia overall, and/or decreasing pneumococcal CAP and/or IPD, and/or need for patient hospitalization for influenza and pneumonia, and patient mortality [43–51]. With regard to the specific pneumococcal vaccines having some, or all, of these effects potential efficacy has been documented for the early whole cell killed bacterial vaccines containing pneumococci and for the more recent pneumococcal polysaccharide vaccine and the pneumococcal conjugate vaccines, in studies in children and/or adults, including the elderly [43–51]. It is therefore not surprising that

several investigators have stressed the potential importance of pneumococcal vaccination as part of pandemic influenza preparedness [45–47, 50, 52].

Cardiac complications of CAP

There is increasing awareness of the possible occurrence of cardiovascular complications in patients hospitalized with CAP [reviewed in 53–57]. Musher and colleagues were the first authors to describe the occurrence of acute cardiac events in patients with pneumococcal pneumonia in a retrospective record review [58]. They identified cases that had had an arrhythmia and/or an acute myocardial infarction (AMI) and/or new or worsening congestive heart failure (CHF). These investigators noted that 33 of 170 patients (19.4%) admitted to hospital for pneumococcal CAP had one or more of these cardiac events. Overall, 12 cases had AMI, 8 had new onset of atrial fibrillation or ventricular tachycardia, and 13 cases had new or worsening CHF, while a number of cases had more than one of these cardiac events. Importantly, the occurrence of cardiac events in patients with pneumococcal CAP was associated with a higher mortality compared with those cases that had no such cardiac events ($p<0.008$) [58]. Recent research has focused on the mechanisms by which pneumococcal infections may precipitate these acute cardiovascular events, providing insights which may enable the development of counteracting strategies. Furthermore, studies have also been initiated to determine whether pneumococcal vaccination may reduce the risk of cardiac events in patients with CAP. One such study suggested that vaccination with the pneumococcal polysaccharide vaccine, PPV23, was associated with a substantial reduction in acute cardiac events in patients with CAP, although sensitivity analyses suggested that much of this benefit may be due to confounding factors, most likely the so-called

“healthy vaccine effect” [59]. Further studies on the potential cardiac protective effects of the pneumococcal conjugate vaccine 13 (PCV 13), which is now licensed for use in adults, still needs to be undertaken.

Biomarkers of disease severity and mortality

In addition to guiding antibiotic therapy, the traditionally used host-derived biomarkers of inflammation and inflammation-associated organ damage, C-reactive protein (CRP) and procalcitonin (PCT), may also help predict disease severity [60]. Other biomarkers of disease severity, some of which are also predictive of myocardial injury, include midregional proadrenomedullin, copeptin, prohormone forms of atrial natriuretic peptide, and cortisol [60–67]. In this context, it is noteworthy that Chang *et al.* reported that “elevated N-terminal B-type natriuretic peptide is a strong predictor of mortality from CAP independent of clinical prognostic indicators“ [68]. However, in a recently published systematic review covering 24 articles and 2 databases from 1069 reviewed abstracts, encompassing 10,319 patients, the utility of measurement of these biomarkers in predicting CAP-related mortality, although demonstrating moderate-to-good accuracy, was not superior to that of established clinical disease severity scores (pneumonia severity index/PSI and CURB-65) [69]. The order or predictive accuracy of these various biomarkers was midregional proadrenomedullin > prohormone forms of atrial natriuretic peptide > cortisol > PCT copeptin > CRP [69].

Role of pneumolysin in the pathogenesis of severe pneumococcal disease

The following section overviews the proposed role of Ply in the pathogenesis firstly of severe CAP, identifying early and later occurring immunosuppressive and pro-

inflammatory phases respectively. This followed by a consideration of the involvement of the toxin in the pathogenesis of CAP-associated myocardial injury.

Early immunosuppressive phase

Pneumolysin (Ply), the major protein virulence factor of the pneumococcus, is a member of the family of microbial, cholesterol-binding, pore-forming toxins, and possesses both cytotoxic and pro-inflammatory properties [70]. The toxin is located in the cytoplasm of the pneumococcus, as well as on the cell-wall, and is released extracellularly following autolysis of the pathogen during the later stages of growth [70, 71]. The key role of Ply in the pathogenesis of pneumococcal pneumonia was clearly demonstrated in two earlier pioneering experimental studies by Feldman *et al.* [72, 73]. In the first of these, exposure of isolated strips of human nasal ciliated epithelium to Ply resulted in ciliary slowing and epithelial disruption, activities of the toxin which favor colonization of the respiratory tract by the pneumococcus [72]. In the second study, these authors observed that injection of recombinant Ply into the apical lobe bronchi of rats resulted in the development of a severe lobar pneumonia, which was restricted to the apical lobe, and comparable in respect of histological changes and severity with that induced by the inoculation of intact, viable pneumococci [73].

Subsequent studies using murine models of experimental pneumococcal lung infection confirmed the key involvement of Ply in the pathogenesis of IPD. Using *ply* gene-knockout mutants or other strategies to neutralize the toxin, these studies demonstrated the role of Ply in promoting: i) colonization of the nasopharynx; ii) bacterial survival, proliferation and extra-pulmonary dissemination; iii) an

exaggerated inflammatory response characterized by pulmonary influx of neutrophils, in the setting of prominent histopathological changes in the lung; and iv) increased mortality [74–79].

The role of Ply in promoting bacterial survival and proliferation following invasion of the lungs is characterized by an early immunosuppressive phase. This is due to the cytotoxic effects of Ply on resident alveolar macrophages in particular, as well as on pulmonary dendritic cells, resulting in cell death due to induction of apoptosis or necroptosis [80–84]. In the case of alveolar macrophages, the healthy lung is populated by resident macrophages with a predominantly anti-inflammatory M2 phenotype [85, 86]. The M2 phenotype is maintained, at least in part, through expression of the transcription factor, interferon regulatory factor 3 (IRF3) and activation of the phosphatidylinositol-3-kinase/protein kinase B (Akt) pathway [87, 88]. Although these cells may initially restrict the intrapulmonary and extrapulmonary spread of the pneumococcus via phagocytosis and exposure of the pathogen to microbicidal proteins in phagolysozomes [80], their antimicrobial potential is limited. This results from attenuation of the capacity of these cells to generate antimicrobial reactive oxygen and nitrogen species (ROS/RNS), as well as apparent failure of activation of the NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome [89–91], a key event in the control of the pneumococcus by various types of inflammatory cells [92–97]. Given the very small intracellular volume of macrophages, Ply released from disintegrating bacteria within phagolysozomes may access the cytosol, reaching concentrations high enough to induce cell death by the aforementioned mechanisms [77, 79, 81, 98, 99]. Thereafter, Ply released by surviving, proliferating extracellular bacilli facilitates extrapulmonary dissemination of

the pathogen via toxin-mediated disruption of lung epithelium and endothelium [76, 77].

Pro-inflammatory phase

This initial immunosuppresive phase of invasive pneumococcal disease is followed by an exaggerated inflammatory response characterized by an early influx of neutrophils followed by monocytes/macrophages with an inflammatory phenotype, and latterly, by T-lymphocytes [75]. As opposed to being protective, however, these Ply-driven inflammatory responses intensify the risk of inflammation-mediated tissue damage and spread of the pneumococcus via several mechanisms including poorly controlled activation of: i) complement ; ii) the NLRP3 inflammasome; iii) neutrophil extracellular trap formation; and iv) platelets.

Ply-mediated complement activation

Ply promotes inappropriate activation of both the classical and lectin-binding pathways of complement activation [70, 100], not only interfering with opsonophagocytosis of the pneumococcus through depletion of complement, but also driving misdirected influx and activation of inflammatory cells via generation of complement-derived chemoattractants. Excessive release of indiscriminate ROS and proteases from these cells exacerbates damage to epithelium and endothelium mediated by the direct cytotoxic actions of Ply [76, 77].

Ply-mediated activation of the NLRP3 inflammasome

As mentioned above, activation of the NLRP3 inflammasome appears to be a key event in the eradication of the pneumococcus by the pulmonary innate immune

system. In this context, activation of NLRP3 by Ply in various cell types such as M1 monocytes/macrophages, dendritic cells, neutrophils in the lungs, as well as microglia in the central nervous system, is protective [92–97]. The triggering event is potassium efflux from these cells consequent to the pore-forming activity of the toxin [93, 101]. This, in turn, leads to caspase-1-dependent proteolytic processing and extracellular secretion of the pro-inflammatory cytokines, interleukin (IL)-1β and IL-18 [102]. However, in the setting of a high bacterial load and excessive production of Ply, consequent unrestrained activation of the NLRP3 inflammasome intensifies the potential threat of inflammation-mediated bystander tissue damage and organ dysfunction [77, 103].

Ply-mediated neutrophil extracellular trap formation

Neutrophil extracellular traps (NETs) constitute a highly-conserved mechanism of phagocyte-mediated antimicrobial activity which extends beyond the lifespan of these cells. Although originally described in neutrophils [104], the process of NETosis is not unique to these cells, having been described in various other cell types of the innate immune system [105]. NETosis is activated following exposure of the cells to various receptor-dependent and -independent signals, some of which initiate a suicidal NETosis and others a vital NETosis, through a series of highly coordinated events, which are incompletely understood. The end result is the release of NETs, comprising an extracellular mesh of decondensed chromatin formed by hypercitrullination of histones, which is heavily impregnated with cytosolic and granule-derived antimicrobial proteins. These NETs entrap and restrict the dissemination of microbial pathogens, which, in some cases, are also killed by NET-bedecked antimicrobial proteins [105].

In this context, it is noteworthy that Ply, at low, non-cytolytic concentrations has been reported to induce vital NETosis *in vitro* [106]. Although this mechanism is potentially protective, the pneumococcus appears to be particularly adept at evading NETs, due in large part to the repulsive actions of the polysaccharide capsule [107] and the activity of the NET-degrading endonuclease, EndA [108]. Although speculative, the pneumococcus may therefore utilize Ply to promote NETosis, which is subsequently subverted by the pathogen, further increasing the risk of inflammation-associated damage to pulmonary epithelium and endothelium, resulting from the cytotoxic actions of the histone components of NETs [109, 110].

Ply-mediated activation of platelets

In addition to their classical pro-thrombotic activities, there is currently increasing awareness of the role played by platelets in orchestrating inflammatory responses, particularly those involving neutrophil/endothelial interactions [111]. Ply appears to modulate platelet activation both indirectly, via activation of the generation of the highly pro-inflammatory bioactive lipid, platelet-activating factor (PAF), by structural and inflammatory cells, as well as by direct actions of the toxin on platelets.

The involvement of PAF in the pathogenesis of Ply-mediated acute lung injury (ALI) was demonstrated by Witzenrath et al., who used an experimental model in which isolated, ventilated, blood-free-perfused lungs from wild-type and PAF receptor gene knockout mice were exposed to the toxin [112]. Exposure of lungs from wild-type mice to Ply resulted in development of pulmonary hypertension and microvascular leakage, both of which were attenuated by pre-treatment of the lungs with a PAF receptor antagonist. These harmful effects of the toxin were also diminished in the

lungs of the PAF receptor gene knockout mice [112]. Although the cellular source was not identified, the authors proposed that PAF in their experimental setting originated from Ply-exposed endothelial cells. This, in turn, led to PAF-mediated autocrine production of thromboxane A_2 (TxA_2), a potent mediator of both vasoconstriction and microvascular platelet aggregation, resulting in pulmonary hypertension and microvascular leakage [112].

Although largely unexplored, infiltrating pulmonary neutrophils also represent a potential source of PAF. In this context, it is noteworthy that neutrophils, unlike alveolar macrophages express high levels of the PAF-generating enzyme, PAF acetylhydrolase [113]. Recently, we have reported that exposure of isolated, human, blood neutrophils to Ply, at concentrations representative of both the experimental and clinical settings [114, 115], caused significant activation of the production of PAF, and, to a lesser extent, T_xA_2 [116]. If operative in the setting of invasive pneumococcal disease, Ply-mediated activation of production of PAF by neutrophils may also contribute to the pathogenesis of acute lung injury (ALI), as well as to the cardiac and other complications, of severe pneumococcal infection.

Direct Ply-mediated homotypic aggregation of platelets represents an additional, also largely unexplored, mechanism of ALI during severe pneumococcal disease. In this context, an earlier study reported that addition of the toxin, as well as several other types of bacterial pore-forming toxins, to undiluted platelet-rich plasma taken from a single human donor, resulted in rapid, marked platelet aggregation comparable in extent to that elicited by the P2Y12 receptor agonist, adenosine 5’-diphosphate [117]. Although the effects of Ply on platelet aggregation were convincing, the

authors did not investigate the mechanisms underpinning platelet activation [117]. More recently, we have observed that exposure of human blood platelets to low, sub-lytic, concentrations of Ply *in vitro* resulted in significant upregulation of expression of the highly pro-inflammatory adhesion molecule, CD62P (also known as P-selectin), a recognized mediator of homotypic platelet aggregation [118]. The mechanisms underpinning these events involved Ply-mediated sub-lytic pore formation, influx of extracellular Ca^{2+}, a key event in platelet activation [118], and Ca^{2+} -dependent mobilization of CD62P-expressing platelet α-granules [119].

Importantly, platelet-neutrophil heterotypic aggregation also involves adhesive contact between CD62P on platelets and its counter receptor, P-selectin glycoprotein ligand-1 (PSGL-1), on neutrophils, interactions which in experimental systems appear to promote both neutrophil migration and NETosis [111, 120], also contributing, to the development of ALI [121].

The aforementioned description of the apparent role of Ply in the immuno-pathogenesis of severe pneumococcal infection and associated ALI is derived from a substantial body of evidence consistent with the changing roles of the toxin throughout the course of infection as summarized in Figure 1. These include initial suppression of innate pulmonary host defenses, followed by the transition to a predominantly pro-inflammatory role, with both phases contributing not only to the survival, proliferation and extrapulmonary spread of the pneumococcus, but also to the associated organ damage and dysfunction.

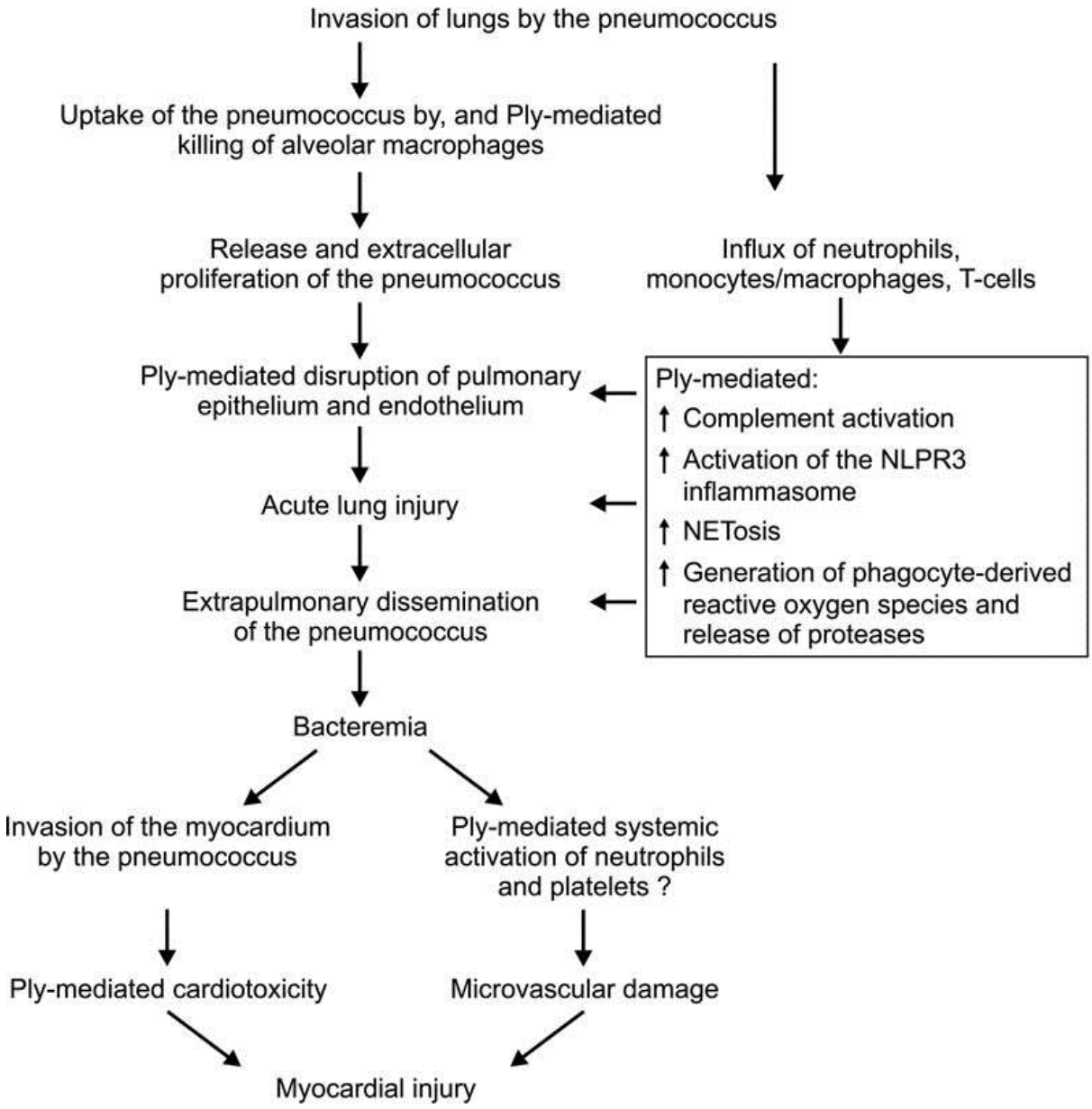

Fig. 1 Summary of the proposed mechanisms by which pneumolysin (Ply) contributes to the pathogenesis of lung and myocardial injury during severe pneumococcal infection.

Ply-mediated cardiac dysfunction

Some aspects of this section of the current review have recently been described elsewhere [122] and these are updated here. Notwithstanding its role in the pathogenesis of ALI, evidence, largely derived from experimental studies, is also consistent with the involvement of Ply in the pathogenesis of the well-recognized occurrence of cardiac damage and dysfunction associated with IPD described

above. In this setting, both direct Ply-mediated cardiotoxicity, as well as inflammation-related mechanisms, have been implicated in the etiology of injury to the myocardium (Figure 1).

Ply-mediated direct cardiotoxicity

Brown *et al.* recently reported that experimental infection of mice (intraperitoneal) and rhesus macaques (intrapulmonary) with the pneumococcus resulted in bacteremia and translocation of the pathogen into the mycocardium, which was dependent on the expression of the pneumococcal adhesin, choline-binding protein A (CbpA) [123]. This, in turn, resulted in the formation of “unique microlesions that disrupt cardiac function” [123]. These effects were less pronounced following induction of experimental infection with a Ply-deficient strain of the pneumococcus, as well as by prior immunization with a pneumolysoid attenuated with respect to pore-forming activity, clearly implicating Ply in the pathogenesis of cardiac microlesion formation [123]. The clinical relevance of these findings was supported by the detection of similar microlesions in cardiac sections from patients with fatal IPD [123].

The findings of the study reported by Brown *et al.* [123] were confirmed in a later investigation by Alhamdi *et al*. [124], using a murine model of IPD and measurement of circulating cardiac troponins as biomarkers of myocardial injury. IPD was accompanied by the development of acute cardiac damage which was: i) not detectable using Ply-deficient mutants of the pneumococcus; ii) attenuated by co-administration of Ply-neutralizing liposomes; and iii) mimicked by intravenous administration of pure, recombinant Ply [124]. Although the injurious effects of Ply on

the myocardium were attributed to direct cardiotoxicity, other Ply-related, indirect mechanisms may also be operative. These include the release of histones from various cell types following exposure to Ply, including cardiomyocytes, epithelial cells and endothelial cells, which may exacerbate myocardial injury. In this context, histones, via their direct cytotoxic actions, have been described as “novel and important mediators of septic cardiomyopathy” [125, 126]. In addition, the cytotoxic actions of histones, as well as those of Ply, on vascular endothelium, are likely to create a pro-thrombotic environment favoring microvascular coagulation.

Cardiac injury secondary to the pro-inflammatory activities of Ply

The systemic, pro-inflammatory activities of Ply released during bacteremic infection with the pneumococcus may also contribute to the pathogenesis of acute coronary events. Prominent potential mechanisms include activation of both NETosis and platelet aggregation.

As mentioned above, exposure of neutrophils to Ply *in vitro* has been reported to trigger NETosis [106]. Systemic, excessive activation of NETosis has, in turn, been linked to the pathogenesis of acute myocardial infarction through various pro-thrombotic mechanisms [127–130]. These include expression of functional tissue factor by NETs [129], as well as the injurious effect of NET-associated histones on vascular endothelium, resulting in the release of von Willebrand factor [130]. In addition, a murine model of histone-induced cardiotoxicity has revealed that sequential neutrophil accumulation, NET formation and thrombosis in the pulmonary microvasculature is the cause of right ventricular dysfunction [126].

Notwithstanding neutrophil activation, Ply-mediated, direct activation and homotypic aggregation of platelets via upregulation of expression of CD62P and other adhesion molecules, as mentioned above, represents an additional, potential mechanism of microvascular obstruction and myocardial injury during severe pneumococcal disease [117, 119, 131].

Empiric antibiotic treatment for CAP

There remains considerable debate as to what constitutes appropriate antibiotic therapy for patients with CAP in the different clinical settings (outpatient, inpatient, ICU), as well as the importance of early initiation of antibiotic treatment [132]. Various international guidelines have been developed, the most commonly quoted ones being those of the Infectious Diseases Society of America/American Thoracic Society (which are currently being updated) [133] and the European Respiratory Society/European Society for Clinical Microbiology and Infectious Disease [134]. Recommendations from the former guideline include the use of a macrolide or doxycycline in outpatients who are previously healthy and have not recently received an antibiotic, while in cases with underlying co-morbidity and/or use of antibiotics either fluoroquinolone monotherapy or beta-lactam-macrolide combination is recommended [133]. For inpatients, not in the ICU, fluoroquinolone monotherapy or beta-lactam-macrolide combination therapy is recommended, and for ICU cases a beta-lactam plus either a macrolide or a fluoroquinolone is recommended [133]. The European guideline recommends agents such as a beta-lactam antibiotic or tetracycline for outpatients with a lower respiratory tract infection, whereas for in-patients, particularly those with severe CAP, the use of a beta-lactam-macrolide

combination or fluoroquinolone monotherapy features prominently among the recommendations [134].

One area of considerable ongoing debate is the issue of whether combination antibiotic therapy, most commonly the use of a beta-lactam plus a macrolide, is required in patients with severe CAP and in particular severe pneumococcal CAP. One of the earliest studies in patients with bacteremic pneumococcal pneumonia was that of Waterer and colleagues, which documented that monotherapy appeared to be suboptimal in severe cases with a Pneumonia Severity Illness (PSI) score of > 90 [135]. Baddour and colleagues, in a prospective, multicenter, international study, documented that combination antibiotic therapy was associated with a lower mortality in severely ill cases with IPD (Pitt bacteremia score >4) [136]. One of the most recent studies in severe pneumococcal pneumonia, which was a matched case control study of two prospectively recorded ICU cohorts in Europe, documented on multivariate analysis that ICU mortality was decreased with early initiation of antibiotics (OR 0.36; 95% CI 0.15-0.87) and use of combination antibiotic therapy (OR 0.19; 95% CI 0.70-0.51) [137]. A detailed literature review confirmed that combination antibiotic therapy, especially the use of a beta-lactam-macrolide combination, appeared to be associated with a lower mortality among severe cases of CAP that required admission to ICU. In addition, there appeared to be a better patient outcome, although not always a lower mortality, in non-ICU patients with CAP who had risk factors for a poor outcome or bacteremic pneumococcal pneumonia [138]. A recent update on macrolide combination therapy recorded that many studies that have documented improved mortality with the use of combination therapy, have also described the possible mechanisms underpinning the benefit of macrolide use

in severe CAP, most importantly the immunomodulatory effects of these agents [139].

Adjuvant strategies targeting pneumolysin

These have also been covered in a recent review [122], and this topic is extended and updated here. Ply-directed therapeutic strategies which have demonstrated protective efficacy in murine models of experimental IPD include: i) intravenous administration of a cocktail of 3 murine monoclonal antibodies directed against different epitopes on the Ply molecule [140]; ii) intravenous administration of cholesterol/sphingomyelin-enriched, Ply-neutralizing liposomes [141]; iii) similarly, by intracutaneous administration of liposomes enriched with the phytosterol, β-sitosterol, which mimics the Ply-binding activity of cholesterol [142]; and iv) the same authors who reported on the Ply-neutralizing actions of β-sitosterol have also reported that verbascoside, a plant-derived phenylpropanoid glycoside, also targets Ply, and, when administered subcutaneously, protects mice against lethal infection with the pneumococcus [143]. Although interesting, impracticalities in the clinical setting such as expense, dosage, timing and routes of administration, together with lack of phase II/III clinical evaluation, restricts the therapeutic application of these Ply-neutralizing strategies.

Currently, macrolides in particular, and possibly statins, appear to be the most promising agents with respect to therapeutic targeting of Ply. Notwithstanding secondary anti-inflammatory activity, which may counter the pro-inflammatory actions of Ply, macrolides and macrolide-like antimicrobial agents effectively inhibit the synthesis of Ply by both macrolide-susceptible and –resistant strains of the

pneumococcus both *in vitro* [144–147], as well as in animal models of experimental infection [114, 144]. These activities, which result from the predominantly bacteriostatic, inhibitory effect of macrolides on bacterial protein synthesis, are not shared by bactericidal antibiotics, some of which may even potentiate the release of Ply [148]. Notwithstanding secondary, immunomodulatory properties, these inhibitory effects of macrolides on the synthesis of Ply by the pneumococcus are also likely to contribute to the utility of these agents in the adjunctive therapy of severe CAP [122].

On a cautionary note, however, some concerns have been raised in relation to the inclusion of macrolides in the antimicrobial/adjunctive therapy of CAP due to the existence of data linking these agents to an increased risk of cardiovascular disease, albeit by poorly characterized mechanisms [recently reviewed in 149]. These concerns may, however, be “overstated” according to the findings of a very recent population-based, retrospective cohort study conducted in Canada over the period 2002-2013 [150]. The authors compared the risk for development of ventricular arrhythmia in 2 matched groups of adults aged >65 years within 30 days of receiving a new prescription for either an orally administered macrolide (azithromycin, clarithromycin, or erythromycin, n= 288,515) or a non-macrolide antibiotic not associated with risk of cardiovascular disease (amoxicillin, cefuroxime, or levofloxacin, n=288473) [150]. The authors reported that “compared with non-macrolide antibiotics, new use of macrolide antibiotics was associated with a similar 30-day risk of “a hospital encounter” with ventricular arrhythmia (0.03% v. 0.03%) and a slightly lower risk of 30-day all-cause mortality (0.62% v. 0.76%)” [150].

The putative role of statins in protecting against the development of acute coronary events in severe CAP is a topic of considerable current interest and has recently been reviewed elsewhere [55] and is discussed only briefly here. Notwithstanding secondary anti-inflammatory activity, the primary cholesterol-lowering actions of these agents consequent to their inhibitory effects on hydroxyl-methylglutaryl-coenzyme A reductase in eukaryotic cell membranes may antagonize the binding of Ply. Although not yet demonstrated in neutrophils or platelets, statins, specifically simvastatin, but also primvastatin, have been reported to protect both isolated human airway epithelial cells [151] and microvascular endothelial cells [152] against the cytotoxic actions of Ply. The latter study also documented a survival benefit of administration of simvastatin to pneumococcus infection-prone sickle-cell disease mice experimentally infected with the pathogen [152].

In addition to targeting Ply, macrolides, as mentioned above, as well as statins, possess a range of other anti-inflammatory activities encompassing various cell types and their inflammatory mediators, underscoring the apparent versatility of these agents in the adjunctive therapy of CAP [reviewed in 55 and 153]. Other types of anti-inflammatory agent which show considerable promise in the adjunctive therapy of CAP include corticosteroids [154, 155] and various categories of anti-platelet agents [55].

Mortality in pneumococcal pneumonia

The mortality of pneumococcal pneumonia still remains high and is dependent on three factors, namely host factors, microbe factors and factors related to antibiotic treatment. In the case of bacteremic infections mortality has been variously reported as ranging from 10% to as high as 36% and has changed very little in the past

several decades [10, 156]. In one multicenter study of severe pneumococcal pneumonia, in cases admitted to an ICU, hospital mortality was 28.8% [157]. In that study host factors, including age (OR 1.05; 95% CI 1.02-1.08), and male gender (OR 2.83; 95% CI 1.16-6.91), were independent risk factors for mortality. While that study did not document associated co-morbidities to have an influence on outcome, other studies have documented that in IPD occurring in patients with underlying risk factors there is an increased risk of hospitalization and death [22]. Tobacco smoking has been documented to be associated with an increased risk of death in patients with pneumococcal pneumonia [26]. Similarly, alcohol abuse has been associated with increased in-hospital mortality in patients with pneumococcal CAP [28].

With regard to the CAP pathogens, particularly the pneumococcus, it has been documented in a number of studies that the current incidence and levels of antibiotic resistance have relatively little impact on the outcome of CAP in patients treated with guideline concordant therapy [14, 15]. However, it is recognized in some studies that there is an association between the different pneumococcal serotypes and risk of death from bacteremic pneumococcal pneumonia [158, 159].

The impact of antibiotic treatment on outcome of pneumococcal CAP was discussed more fully above, but early initiation of antibiotic therapy and use of combined beta-lactam-macrolide therapy have been shown to be associated with decreased mortality in patients with severe pneumococcal CAP [137].

Lastly, it has been noted in many studies that the long-term prognosis of patients recovering from CAP is impaired for a number of reasons, including the presence of

underlying comorbidities that put them at risk of CAP in the first instance and also because of the occurrence of cardiovascular events [160].

Conclusions

Given the ongoing threat posed by pneumococcal CAP worldwide, particularly that associated with progressive population ageing in Western Europe and the USA, optimizing prevention and early recognition of those at highest risk for development of life-threatening complications, represent significant challenges. In this context, improving pneumococcal and influenza immunization rates in older adults, together with the identification of systemic biomarkers which accurately predict those at highest risk for a poor outcome who would benefit from early implementation of adjuvant therapies, are priorities.

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