

---

# The Global Burden of Community-Acquired Pneumonia, Encompassing Invasive Pneumococcal Disease and the Prevalence of Its Associated Cardiovascular Events, With a Focus on Pneumolysin and Macrolide Antibiotics in Pathogenesis and Therapy

---

[Ronald Anderson](#) \* and [Charles Feldman](#)

Posted Date: 30 May 2023

doi: 10.20944/preprints202305.2086.v1

Keywords: community-acquired pneumonia; cardiovascular events; dendritic cells; macrolides; macrophages; mannose receptor C-type1; platelets; pneumolysin; pro-inflammatory cytokines; *Streptococcus pneumoniae*.



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Review

# The Global Burden of Community-Acquired Pneumonia, Encompassing Invasive Pneumococcal Disease and the Prevalence of Its Associated Cardiovascular Events, with a Focus on Pneumolysin and Macrolide Antibiotics in Pathogenesis and Therapy

Ronald Anderson <sup>1,\*</sup> and Charles Feldman <sup>2,\*</sup>

<sup>1</sup> Department of Immunology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

<sup>2</sup> Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand Medical School, 7 York Road, Parktown, Johannesburg 2193, South Africa

\* Both authors contributed equally to the compilation of this manuscript.

\* Correspondence: ronald.anderson@up.ac.za; +27-319-2425; +27-323-0732

**Abstract:** Despite innovative advances in anti-infective therapies and vaccine development technologies, community-acquired pneumonia (CAP) remains the most persistent cause of infection-related mortality globally. Confronting the ongoing threat posed by *Streptococcus pneumoniae* (the pneumococcus), the most common bacterial cause of CAP, particularly to the non-immune elderly, remains challenging due to the propensity of the elderly to develop invasive pneumococcal disease (IPD), together with the predilection of the pathogen for the heart. The resultant development of often fatal cardiovascular events (CVEs), particularly during the first seven days of acute infection, is now recognized as a relatively common complication of IPD. The current review represents an update on the prevalence and types of CVEs associated with acute bacterial CAP, particularly IPD. In addition, it is focused on recent insights into the involvement of the pneumococcal pore-forming toxin, pneumolysin (Ply), in subverting host immune defenses, particularly the protective functions of the alveolar macrophage during early-stage disease. This, in turn, enables extra-pulmonary dissemination of the pathogen, leading to cardiac invasion, cardiotoxicity and myocardial dysfunction. The review concludes with an overview of the current status of macrolide antibiotics in the treatment of bacterial CAP in general, as well as severe pneumococcal CAP, including a consideration of the mechanisms by which these agents inhibit the production of Ply by macrolide-resistant strains of the pathogen.

**Keywords:** community-acquired pneumonia; cardiovascular events; dendritic cells; macrolides; macrophages; mannose receptor C-type1; platelets; pneumolysin; pro-inflammatory cytokines; *Streptococcus pneumoniae*.

## 1. Introduction: Burden of Community-Acquired Pneumonia (CAP)

Community-acquired pneumonia (CAP) remains the leading infectious disease cause of death worldwide, with considerable impact on the healthcare system; furthermore, despite several advances in diagnosis, management, and prevention of disease, there remains several unmet needs with regard to this infection [1]. While there appears to be a substantial decline in the proportion of *Streptococcus pneumoniae* microorganisms causing CAP in North America, the proportion of this microorganism as a cause in Europe, and other areas of the world, remains much higher, possibly related, it has been said to differences in pneumococcal vaccination strategies and smoking habits in different regions [2,3]. Nevertheless, in a more recent systematic review of CAP in adults, the most common causative pathogens were noted to be *S. pneumoniae* (pneumococcus) and *Haemophilus*

*influenzae* [3]. However, the authors did note that with increasing use of polymerase chain reaction (PCR) testing for diagnosis, the number of cases of documented causes of CAP has increased, particularly among the viruses, such that the proportions of all bacteria have diminished [3]. The global burden of disease study on lower respiratory tract infections (LRTIs) indicated that in 2016, a total of 2 377 697 (2 145 584-2 512 809) deaths occurred from LRTIs in people of all ages, with 1 080 958 deaths (943 749-1 170 638) in adults over 70 years of age [4]. *Streptococcus pneumoniae* was the leading cause of LRTI morbidity and mortality globally, causing more deaths than all other etiologies combined in 2016 (*Haemophilus influenza* type b, influenza virus and respiratory syncytial virus). Progress has been made in reducing the burden of LRTIs, but this has not been equal among all regions and more effort is required, particularly in the case of the elderly. While the prevalence of invasive pneumococcal disease (IPD) was noted to decrease worldwide during 2020 and early 2021, thought to be due to the introduction of non-pharmaceutical interventions to prevent spread of the COVID-19 pandemic, some countries noted IPD levels returning to, and even exceeding, seasonal levels later in 2021 [5–7].

## 2. Cardiovascular Complications in Patients with CAP

Considerable research has been undertaken in the past nearly two decades into the occurrence of cardiovascular events (CVE) in patients with infection, particularly in cases with community-acquired pneumonia, and more recently COVID-19. Early studies suggested that incident cardiac events in patients with CAP were common, were associated with increased short-term mortality and that older age, nursing home residence, pre-existing cardiovascular disease and pneumonia severity were factors associated with their occurrence [8]. A more recent systematic review and meta-analysis of observational studies documenting selected CVE in patients with CAP indicated that cardiac complications occurred in 13.9% (95% CI 6.7-12.2), acute coronary syndromes (ACS) in 4.5% (95% CI 2.9-6.5), heart failure in 9.2% (95% CI 6.7-12.2) arrhythmias in 7.2% (95% CI 5.6-9.0), and stroke in 0.71% (95% CI 0.1-3.9) [9]. After adjusting for confounders, CVEs occurring in patients with CAP were independently associated with increased risk for short-term mortality. In a study from the FADOHCECAP Study Group [10] 1266 patients with CAP were enrolled of whom 23.8% experienced a least one CVE.

## 3. Cardiovascular Events in Patients with Pneumococcal CAP

The first study specifically investigating the association between acute cardiac events and pneumococcal pneumonia indicated that this infection was a significant risk for acute cardiac events, such as myocardial infarction, serious arrhythmia or new or worsening congestive heart failure, which substantially increased mortality [11]. Subsequent studies indicated the following; i) in hospitalized patients with CAP and subsequent CVEs, ACS was more common in those with pneumococcal versus non-pneumococcal CAP, but there was no difference in rates of arrhythmias, heart failure or 1-year mortality [12]; ii) in a study assessing whether pneumococcal bacteremia was associated with CVEs in adults hospitalized with pneumococcal CAP, it was documented that pneumococcal bacteremic CAP was associated with a higher rate of CVEs compared with non-bacteremic pneumococcal CAP, driven primarily by higher rates of new onset arrhythmias 86 (9.3%) versus 64 (5.5%);  $p = 0.01$  [13]; iii) in a retrospective study of 1739 adults hospitalized with pneumococcal CAP, ACE occurred in 304 (17.5%) with older age, pre-existing heart conditions, pneumococcal bacteremia, septic shock on admission and high-risk pneumonia identified as risk factors [14]; iv) in a retrospective cohort study of ACE following pneumococcal infection, in which these patients were matched with non-infected patients for each CVE, pneumococcal infections were associated with an elevated risk of subsequent stroke and atrial fibrillation, while effects on short-term health also increased mid-term to long-term susceptibility to CVEs [15]; and v) in a prospective, multi-center cohort study in hospitalized adults with CAP, age, smoking, chronic heart disease, initial severity of infection and pneumococcal infections were risk factors for both early and late CVEs and were associated with increased mortality risk during the diagnosis of CAP and after the episode of pneumonia [16]. One recent study reviewed the data that underlines the damage that occurs during acute pneumococcal infection that can result

in long-term sequelae, including reduction in quality of life and life expectancy in pneumococcal disease survivors [17]. While one earlier study [14] documented no apparent relationship between serotypes or clonal complexes and ACE, a more recent study suggested that serotypes 3 and 9 are more commonly associated with major adverse cardiac events [18].

The two main putative mechanisms that have been implicated in the pathogenesis of myocardial injury in severe pneumococcal pneumonia are the cardiotoxic affects of the pneumococcal pore-forming cytolysin, pneumolysin (Ply), and other pneumococcus-derived products, as well as pro-thrombotic effects involving several pneumococcal virulence factors, including pneumolysin [17,19]. Use of agents active against pneumolysin, or prevention of pneumococcal infections with vaccination, may be strategies to further reduce the impact of pneumococcal CAP and its associated CVEs.

While there need to be studies regarding the potential benefits of influenza and pneumococcal vaccination (PV) against CVEs occurring in patients with influenza and pneumococcal infections, vaccination of patients with underlying cardiac disease could be cost-effective in improving clinical outcomes in those patients [20]. There is evidence for influenza vaccines in protecting against mortality, ACS, and hospitalization in patients with coronary heart disease and CHF; however, the data for PV are generally contradictory. A recent systematic review and meta-analysis to assess the association between recipients of PV and mortality/CV outcomes among patients with and without established CVD documented that PV decreased risk of all-cause mortality and myocardial infarction [21]. Clearly, future randomized controlled trials are needed regarding benefit of PV against CVE and mortality in patients with underlying cardiac disease who have CAP [21].

#### **4. Involvement of Pneumolysin-Mediated Immunosuppression and Cytotoxicity in Disease Pathogenesis**

The development of IPD is triggered by the translocation of this dangerous respiratory pathogen from the nasopharynx to the lower airways. Those with co-existent immunosuppression, particularly the non-immunized very young and elderly are at highest risk. In the very early stages of infection, the pneumococcal pore-forming toxin, Ply, plays a key role in disease pathogenesis by subduing pulmonary host defenses and promoting persistence of the pathogen [22]

##### *4.1. Suppression of Alveolar Macrophage Function by Pneumolysin*

In the lower airways, the pneumococcus encounters alveolar macrophages, which have a hybrid M1/M2 phenotype, possibly enabling phenotype switching according to changing inflammatory environments [23]. Studies using isolated human or animal macrophages, as well as murine models of experimental infection, have revealed several mechanisms by which the pathogen promotes immunosuppression necessary for early persistence in the lung. This is likely to be most prevalent in non-immune persons in whom antibody/complement-mediated phagocytosis and macrophage activation is minimal. In this setting of early disease, there is significant dependence on pathogen/macrophage scavenger receptor interactions to mediate pathogen clearance, especially the involvement of the C-type lectin, mannose receptor C-type (MRC-1) [24]. Notably, however, adhesion of Ply-expressing wild-type serotype 4 TR4 and serotype 2 D39 strains of the pneumococcus, but not their corresponding isogenic *ply* gene knockout mutants, to isolated human dendritic cells (DCs) and macrophages, resulted in detrimental, as opposed to protective, consequences for anti-pneumococcal host defenses. These included attenuation of production of the protective, pro-inflammatory cytokines, tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6 and IL-12 [25]. Mechanistically, Ply-mediated suppression of the early innate, protective, pro-inflammatory cytokine response appeared to involve induction of the suppressor of cytokine signaling 1 (SOCS1), with resultant attenuation of expression of pro-inflammatory genes driven by the transcription factors STAT1 (signal transducer and activation of transcription 1) and NF $\kappa$ B (nuclear factor kappa B) [25]. Dysregulation of cytokine synthesis by Ply also resulted in the induction of naïve T cells with an immunoregulatory phenotype, as well as decreased neutrophil accumulation in the lower airways [25]. The immunosuppressive interaction of Ply with MRC-1 expressed on alveolar macrophages was



confirmed in a murine model of experimental lower airways infection in which MRC-1-deficient mice exhibited a significant reduction in bacterial loads.

Although these immunosuppressive effects of Ply/MRC-1 interactions are mediated by extracellular pneumococci, it is noteworthy that this mechanism also enables the pathogen to penetrate MRC-1 expressing M2-type macrophages and DCs concealed in MRC-1-coated endosomes. However, these do not fuse with phagosomes, enabling evasion of innate bactericidal mechanisms, which include exposure to reactive oxygen species (ROS), antimicrobial peptides and proteolytic enzymes, as well as phagosomal acidification, resulting in intracellular survival and persistence of the pathogen.

Very recently, other investigators using isolated human monocyte-derived macrophages and a murine model of experimental lung infection reported very similar findings [26]. These authors observed that exposure of macrophages to three different Ply-expressing wild-type strains of the pneumococcus [serotypes 4 (TIGR4), 3 and 23F] was significantly less effective than exposure to their Ply-deficient variants with respect both to production of the key protective cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and control of experimental infection of the lower airways [26]. These immunosuppressive effects of the Ply-expressing strains of the pneumococcus, seemingly mediated via interaction of the toxin with MRC-1, were confirmed by transcriptomic analysis of pathogen-exposed macrophages. Mechanistically, the immunosuppressive activity of Ply was also found to be associated with induction of SOCS1, resulting in inhibition of translocation of NF $\kappa$ B to the cell nucleus and resultant attenuation of the transcription of pro-inflammatory genes [26].

Age-related loss of efficacy of Ply-triggered macrophage LC3-associated phagocytosis represents an additional mechanism, which may contribute to the vulnerability of the elderly for development of severe pneumococcal disease [27]. In this context, isolated murine bone-marrow-derived macrophages exposed to Ply-expressing *S. pneumoniae* undergo an autophagy-independent process of post-phagocytic pathogen containment known as microbutule-associated protein 1A/1B light chain=3 (LC3)-associated phagocytosis (LAP), in which LC3 is conjugated with the phagosomal membrane to form so-called "LAPosomes" [27]. Unlike autophagosomes, which have a double membrane structure, LAPosomes have a single membrane modulated by ROS, which regulates the internal pH and oxidative state, possibly contributing to pathogen eradication [27,28]. Nevertheless, the exact mechanism of elimination of LAP-targeted bacterial pathogens remains to be established, as does the exact involvement of Ply in their formation. Notably, however, macrophages from aging mice have been found to have an impairment of LC-3-associated phagocytosis, manifested as defective control of pneumococcal infection. Although of potential importance, the clinical relevance of these findings remains uncertain.

Interestingly, exposure of isolated human monocyte-derived macrophages to Ply has been linked to epigenetic changes in these cells, which are also associated with early immunosuppression [29]. The design of this study involved infecting the cells with either the wild-type D39 strain of *S. pneumoniae*, or the corresponding *ply* gene-deficient mutant, or the reconstituted mutant [29]. Following initiation of experimental infection, the authors monitored the resultant transcriptomic, proteomic and epigenetic landscapes of the macrophages. As described by others [25,26], they demonstrated early differential inflammatory gene expression, which was significantly attenuated in macrophages exposed to the two Ply-replete strains of the pathogen, according to decreased production of TNF- $\alpha$  and IL-6 [29]. In addition, quantitative mass spectrometry revealed extensive histone post-translational modifications, encompassing 94 histone peptide proteoforms, five of which were altered in a Ply-dependent fashion (three increased, two decreased). Although possible associations of these with Ply-mediated attenuation of the innate immune response remain to be conclusively established, it is noteworthy that inclusion of the histone deacetylase inhibitor, vorinostat, mimicked the suppressive effects of Ply on macrophage production of TNF- $\alpha$  [29].

#### 4.2. Dendritic Cells

As alluded to above, exposure of isolated monocyte-derived DCs to a Ply-expressing strain of *S. pneumoniae* resulted in attenuation of production of immunostimulatory cytokines and failure to

activate CD4<sup>+</sup> T cells [25]. More recently, Paulikat *et al.*, have confirmed and extended these findings [30]. Like Subramanian *et al.* [25], these authors compared the expression of biomarkers of immune activation following exposure of human, cytokine-differentiated monocyte-derived DCs to wild-type or *ply* gene-knockout strains of the TIGR4 strain of *S. pneumoniae* [30]. They observed that relative to the responses of the *Ply*-deficient variant of the pathogen, exposure of DCs to the wild-type strain resulted in defective maturation of these cells. This was characterized by attenuation of production of the pro-inflammatory cytokines, IL-6, IL-12 and IL-23, as well as decreased expression of the pro-adhesive, co-stimulatory immune checkpoint molecules CD40, CD80 and CD86 [30]. In addition, dysregulation of DC activation by *Ply* resulted in failure of these cells to activate CD4<sup>+</sup> T cells and possibly, but not proven, induction of Th17 cells [30]. The authors also observed that *Ply*-mediated suppression of the reactivity of DCs was augmented by co-infection with influenza A virus, in keeping with the heightened threat posed by simultaneous infection with these dangerous respiratory pathogens [30].

This section of the review has highlighted the vulnerability of alveolar macrophages, as well as DCs, that populate the lower airways, to *Ply*-mediated early immune dysfunction. These are clearly key events in the pathogenesis of severe pneumococcal disease, which result in early failure to control the infection in susceptible individuals. In this setting of early induction of *Ply*-mediated immunosuppression, cell wall-associated toxin, as opposed to that released extracellularly by the action of *LytA*-mediated autolysis during the late stages of bacterial growth, may also play a significant role [31,32]. As shown in murine models of experimental lower-airways infection, significant growth of *Ply*-expressing strains of the pathogen occurs during the 24-hour period post-induction of infection with the rate of bacterial growth in the lungs dependent on the capsular serotype of the pathogen [33]. Synthesis of *Ply* occurs during the logarithmic phase of growth and the toxin, which lacks an N-terminal secretion signal sequence, is released extracellularly by autolysin-mediated disintegration of the peptidoglycan backbone of the pathogen, significantly increasing the threat of cytotoxicity and pulmonary damage, which favor extra-pulmonary dissemination of the pneumococcus. At these high concentrations of *Ply*, the cholesterol-binding pore-forming activity of the toxin is prevalent, with the potential to damage and kill multiple cell types, not only by necrosis, but also by triggering programmed cell death pathways such as apoptosis and necroptosis depending on cell type [34–36].

## 5. Involvement of Pneumolysin in Extra-Pulmonary Dissemination of the Pneumococcus

The mechanisms involved in the pathophysiology of *Ply*-mediated acute lung injury in the experimental setting, which favor extracellular dissemination of the pneumococcus, have been extensively investigated by Witzenrath *et al.* [37]. Using isolated, ventilated and blood-free murine lungs, these authors explored the effects of a 30-minute challenge with recombinant *Ply* [0.025 – 2.5 micrograms (μg)], administered either by infusion into the pulmonary artery, or intratracheally using aerosolized toxin, on lung microvascular permeability and pulmonary vascular resistance [37]. Administration of *Ply* by both routes caused significant, dose-related increases in capillary permeability, while only infusion of the toxin resulted in significantly increased pulmonary vascular resistance. Immunohistochemistry revealed that *Ply* was present predominantly in the endothelial cells of arterial vessels, which manifested robust vasoconstriction [37]. Additional *in vitro* experiments revealed that exposure of an alveolar epithelial cell line (A549) and human umbilical vascular endothelial cell monolayers to *Ply* (0.1 – 1 μg) resulted in significantly increased permeability of both cell types, which was maximal after 60 min of incubation, while intercellular gap formation was evident in the endothelial cell monolayers [37]. This study indicates that acute pneumococcal pneumonia results in *Ply*-mediated disruption of alveolocapillary barrier function and pulmonary hemodynamics, not only causing respiratory failure, but also enabling extrapulmonary dissemination of both the pathogen and its pore-forming toxin.

A combination of mechanisms triggered by the pore-forming interactions of *Ply* with various types of resident and migratory cells are likely to underpin the pathogenesis of pulmonary damage and dysfunction in severe pneumococcal disease. These include:

- Most prominently, the direct cytotoxic effects of Ply on alveolar epithelial and endothelial cells, resulting from necrosis and necroptosis [38,39]. In this context, the mechanism of necroptotic cell death is independent of the death receptor and is achieved by the initiation of signaling events triggered by Ply-mediated calcium influx and potassium efflux [39,40];
- Induction of the synthesis of platelet-activating factor (PAF) in lung tissue exposed to the toxin, as reported by Witzernath et al., in a follow-up study [41]. Although the cellular origin of PAF was not identified, interactions of this bioactive lipid with endothelial cells was proposed to induce the synthesis of the potent vasoconstrictor, thromboxane A2 [41];
- Pore formation due to exposure of endothelial cells to lower, sub-lytic concentrations of Ply, triggering calcium influx, which causes “adherens function disassembly and cytoskeletal rearrangement to facilitate endothelial cell retraction and increased permeability” [42];
- Involvement of the regulator of endothelial permeability, angiopoietin-2 (ang-2) in Ply-induced endothelial permeability, as reported more recently by Gutbier and other members of the Witzernath research team, using the isolated murine perfused and ventilated lung model, as well as other approaches [43]. These authors reported: i) that perfused and ventilated lungs from ang-2 gene-knockout mice demonstrated reduced permeability on exposure to Ply; ii) exposure of three different types of endothelial cell (human pulmonary microvascular; human umbilical vein; murine lung) to Ply (1 – 2 µg/ml) *in vitro* resulted in dose-related increased production of ang-2, while endothelial barrier integrity was impaired following exposure of the endothelial cells to ang-2; iii) elevated serum concentrations of ang-2 had negative prognostic value in patients with CAP; and iv) ang-1, an antagonist of ang-2, which inhibits calcium influx [44], attenuated Ply-mediated augmentation of endothelial permeability, implicating sub-lytic pore formation and calcium influx in toxin-mediated synthesis of ang-2 [43];
- Platelet activation *per se*, as well as platelet-driven activation of vascular endothelium and infiltrating neutrophils, represent additional contributors to the pulmonary damage and dysfunction during acute pneumococcal pneumonia, which presumably precedes, and appears to be essential, for subsequent cardiac injury. In this context, several inter-linked developments are of particular note. Firstly, the discovery by Le Français *et al.* that the lungs of mice and humans represent a primary site of thrombopoiesis, with the pulmonary circulation of mice accounting for approximately 50% of the body pool of platelets in mice [45], with corresponding estimates of platelet production in the lungs of humans and mice of 7 – 100% [46]. Secondly, Ply has been reported to induce platelet activation in several [47–49], but not all [50], studies, and to promote the formation of neutrophil:platelet aggregates [51] *in vitro*. If operative in the setting of acute pneumococcal pneumonia, these mechanisms are likely to trigger endothelial activation and pulmonary microvascular injury and occlusion. In addition, Ply has been shown to activate the formation of neutrophil extracellular traps (NETs) by isolated human neutrophils [48,52]. Notably, platelet-driven NETosis is a recognized driver of intravascular coagulation and thrombin activation during severe microbial and viral infections [53,54]. It has also been implicated in the pathogenesis of the pulmonary complications of severe COVID-19 [55,56], as well as in the severity of experimental pneumonia in mice [57]. These mechanisms are probable key contributors to the pathogenesis of IPD in humans.

Acting in concert, the aforementioned Ply-mediated mechanisms, which are summarized in Table 1, are likely to inflict the mechanical damage to the lungs, which precedes and is a prerequisite for extrapulmonary dissemination of the pneumococcus, setting the scene for invasion of distal organs. Although alveolar epithelial cells and endothelial cells possess several mechanisms which are mobilized to repair Ply-mediated pores, these mechanisms vary with respect to efficacy and cell type [58–61]. In the case of myeloid cells, pore-damaged membrane is expelled via formation of calcium-dependent vesicles [58,59], while alveolar cells and endothelial cells utilize attenuation of the activities of executioner caspases 3/7 and induction of caveolin-1-mediated endocytosis-dependent mechanisms, respectively [60,61].

**Table 1.** Pneumolysin-mediated mechanisms of immunosuppression and endothelial barrier disruption operative in the lower respiratory tract, which promote proliferation and extra-pulmonary dissemination of the pneumococcus.

<u>Mechanisms of immunosuppression *</u>	<u>Mechanisms of endothelial barrier disruption **</u>
<ul style="list-style-type: none"><li>• Suppression of the protective pro-inflammatory activity of alveolar macrophages and dendritic cells via interaction of Ply with MRC-1 and induction of SOCS1</li><li>• Age-related impairment of LC-3-associated phagocytosis</li><li>• Induction of Ply-associated epigenetic changes following exposure of macrophages to the pneumococcus associated with decreased production of protective, pro-inflammatory cytokines</li><li>• Defective maturation of dendritic cells associated with decreased production of both pro-inflammatory cytokines and expression of co-stimulatory immune checkpoints, as well as failure to activate CD4<sup>+</sup> T cells</li></ul>	<ul style="list-style-type: none"><li>• Direct cytotoxic effects on alveolar epithelial and endothelial cells due to the pore-forming actions of the toxin</li><li>• Induction of synthesis of PAF, which, in turn, activates the synthesis of thromboxane A2 by vascular endothelial cells</li><li>• Upregulation of production of the regulator of vascular permeability, angiopoietin-2, by endothelial cells, which, in turn, causes impairment of endothelial barrier function</li><li>• Induction of platelet activation leading to pulmonary microvascular injury and occlusion due to intravascular formation of platelet:platelet homotypic aggregates, platelet:neutrophil aggregates and platelet-driven NETosis</li></ul>

\*References [58–63]; \*\*References [70–76] and [80–87]; Abbreviations: MRC-1 (mannose receptor C-type 1); SOCS1 (suppressor of cytokine signaling 1); LC3 (microtubule-associated protein 1A/1B-light chain 3); Ply (pneumolysin); PAF (platelet-activating factor); NETs (neutrophil extracellular traps).

6. Role of Pneumolysin in Cardiac Dysfunction during Severe Pneumococcal Disease

Severe pulmonary damage inflicted by Ply, acting in concert with other pneumococcal toxins, especially hydrogen peroxide, enables escape and systemic spread of the pathogen. Invasion of the myocardium, for which the pneumococcus has a notable predilection, represents a significant risk, particularly during the first seven days of development of IPD when the incidence rate ratio peaks at 92.95 (95% CI; 72.17 – 119.71) [62]. The involvement of Ply in the pathogenesis of IPD-associated CVEs has been expertly and convincingly explored in a series of murine and non-human primate experimental infection studies [63–65], which have been collated in several reviews [19,22].

These studies revealed that the initial event in translocation of the pneumococcus to the myocardium is strain-specific and dependent on a high degree of bacteremia [66] and possibly on the level of expression of the ZomB protein, which is a novel positive transcriptional regulator of the *ply* operon [67]. The interaction of the bacterial adhesins, choline-binding protein A (CbpA) and phosphorylcholine with the endothelium of myocardial microcapillaries are key events, which enable translocation of the pathogen from the circulation into the heart tissue [63,64]. The pneumococcus then becomes established in early microlesions, perpetuating its presence via Ply-mediated necroptosis of infiltrating and resident, sentinel cardiac macrophages [68,69]. The resultant immunosuppression enables the formation of mature, persistent intra-cardiac microlesions and transition of the pneumococcus to a biofilm-forming/high Ply-producing phenotype [69]. These events, in turn, inflict impaired cardiomyocyte contractibility and cell death, events which precede severe myocardial dysfunction [70].

Importantly, a recent experimental animal study has highlighted the risk posed by co-infection with influenza A virus (IAV) and IPD on the incidence and severity of cardiac damage and dysfunction. In this study, Platt *et al.* investigated the effects of induction of infection of mice with a mouse-adapted strain of IAV followed 10 days later by challenge with the TIGR4 strain of the pneumococcus [71]. Co-infection with both respiratory pathogens, as opposed to the pneumococcus alone, resulted in a significant increase in the percentage area of the heart, which manifested demonstrable microlesions, as well as a 1000-fold increase in bacterial titers [71]. Mechanistically,



primary infection with IAV resulted in significant upregulation of cardiomyocyte adhesion molecules, specifically the polymeric Ig receptor and fibronectin leucine-rich transmembrane protein 1, which interact with the pneumococcal adhesins PspC and PfbA/PavA/PavB, respectively [70]. Complementary *in vitro* studies revealed that exposure of AC16 cardiomyocytes to the wild-type strain of the pneumococcus resulted in high-level cytotoxicity, which was attenuated by selective knockout of any one of the genes encoding the pneumococcal adhesins, PspA, CbpA or PsrP, as well as that encoding Ply [71]. The involvement of Ply in co-infection-mediated augmentation of cardiotoxicity was strengthened by the observation that pre-treatment of cardiomyocytes with recombinant toxin significantly increased the degree of cellular injury [71].

As with pulmonary damage, a second, possibly interactive, albeit less intensively explored, mechanism of cardiotoxicity, relates to the potential of Ply to promote systemic activation of platelets and neutrophils. These events may result in myocardial microvascular occlusion due to the formation of platelet:platelet homotypic aggregation and platelet:neutrophil heterotypic aggregation, as well as platelet-driven NETosis [19,22].

A third potential mechanism of Ply-mediated cardiotoxicity, albeit unexplored in the setting of experimental pneumococcal infection, involves the interaction of Ply and pneumococcal neuraminidase A (NanA) with high-density lipoproteins [72]. In this study, Syed and colleagues observed that exposure of HDL particles to the combination of Ply and NanA *in vitro* resulted in significant alterations to the sialic acid, protein and lipid composition of the lipoproteins. These alterations in molecular structure were associated with oxidative damage and pro-inflammatory activity, as well as increased complement activity, resulting in increased pro-atherogenic potential [72].

These various mechanisms of Ply-mediated cardiotoxicity are summarized in Table 2.

**Table 2.** Summary of pneumolysin-mediated mechanisms of immunosuppression and cardiotoxicity in the pathogenesis of severe myocardial dysfunction.

Mechanism	Reference
• Ply-mediated suppression of the protective activities of infiltrating and resident sentinel cardiac macrophages via induction of necroptosis following adherence of the pathogen to the vascular endothelium of myocardial capillaries	101,102
• Following invasion of the myocardium the pneumococcus becomes established in intra-cardiac microlesions in which it transitions to a biofilm-forming, high Ply-producing phenotype	102
• Cardiotoxicity results from interaction of the pneumococcal adhesins PspA, CbpA and PsrP with cardiomyocytes, resulting in exposure of these cells to Ply, leading to cell death and myocardial dysfunction	103,104

**Abbreviations:** Ply (pneumolysin); PspA (pneumococcal surface protein A); CbpA (choline-binding protein A); PsrP (pneumococcal serine-rich repeat protein).

The preceding sections of this review, which have focused on Ply, clearly identify this toxin as a key target in the therapy of IPD and protection against development of CV complications. In this context, and in keeping with the recent clinical study by Chowers *et al.* focused on the role of the type of antimicrobial chemotherapy on mortality in bacteremic pneumococcal pneumonia, reinforce the role of macrolide antibiotics in protecting against Ply, which is considered in the following sections [73].

7. Antibiotic Treatment

Regarding antibiotic treatment of CAP, current guidelines indicate that despite concerns of increasing antibiotic resistance among the pathogens, most patients with CAP can currently still be successfully treated with antibiotic regimens that have been used for many decades [74]. One area of

particular concern with regard to antibiotic resistance has been macrolide resistance, which has been shown to be associated with variable, though generally high, rates of resistance among pneumococcal isolates in the United States, such that some authors have suggested that there should be reconsideration of macrolide monotherapy, at least in some areas of the country [75]. Another area of controversy with regard to macrolide use is the need for beta-lactam/macrolide (BM) combination therapy in outpatients with comorbidities, or inpatients with risk factors for antibiotic resistant pathogens, or severe CAP, versus the use of fluoroquinolones (FQs) alone, as per current guidelines [74,76,77]. Cilloniz and co-authors conducted a retrospective, observational study of adults hospitalized for CAP who had positive cultures for *S. pneumoniae*, and found no evidence that patients infected with macrolide-resistant pathogens were either more severely ill or had worse outcomes if treated with guideline compliant therapy [78]. Despite increasing resistance, based on the evidence from the Cilloniz et al. study, as well as other studies, many authors continue to recommend the use of macrolides, usually as part of combination therapy, because of their non-antimicrobial anti-inflammatory effects [76,77,79]. Authors have also suggested that patients with COVID-19 pneumonia and suspected co-infection with CAP, especially if severe, should receive BM therapy as for CAP alone [80,81].

## 8. Benefits of Beta-Lactam/Macrolide Therapy in Patients with CAP

Several more recent studies have evaluated the potential benefits of BM combination therapy in patients with CAP, compared with beta-lactam monotherapy (BL) or FQ monotherapy.

### 8.1. Non-ICU Hospitalized CAP

A systematic review and meta-analysis, as well as a network meta-analysis, indicated that FQ monotherapy had similar efficacy and safety compared with BL with or without a macrolide [82,83]. Furthermore, in a retrospective cohort study there was no difference in 30-day readmissions in patients with CAP who received FQ versus BM [84]. In contrast, in a retrospective cohort study in Korea, BM combination therapy among patients with a CURB-65 score  $\geq 2$ , the 30-day survival rate was higher in the BM combination than in the BL monotherapy-treated group (93.3% versus 88.5%;  $p = 0.009$ ), but this benefit was only seen in autumn, but not spring [85]. In a cohort of patients with CAP of moderate severity (non-ICU cases), a simple decision tree (patients without CVD, or patients with respiratory comorbidities and high leukocyte counts in respiratory secretions) distinguished patients who would benefit from the addition of a macrolide to a BL agent, leading to a 27% (OR 1.83, 95% CI 1.48-2.27;  $p < 0.001$ ) reduction in mortality, compared to standard of care [86]. This outcome benefit needs a randomized controlled trial to confirm the findings.

### 8.2. Moderate to Severe CAP, including ICU Patients

Three studies evaluated combination therapy versus BL or FQ monotherapy. The first study showed that the efficacy of ceftriaxone combination therapy was similar to that of FQ monotherapy, as well as being associated with lower drug-related adverse events [87]. The second study demonstrated no differences in short- or long-term outcomes in CAP patients treated with either BM combination therapy or FQ monotherapy [88]. The third study showed that compared to BL monotherapy, treatment with azithromycin plus BL had no benefit on the in-hospital mortality of mechanically ventilated patients with CAP and CAP-associated ARDS [89].

In contrast to the former studies, several additional studies have shown benefit of BM combination antibiotic therapy over other therapies in hospitalized cases with CAP, including those admitted to ICU. One prospective, observational study of hospitalized cases with CAP of known microbial cause indicated that BM combination therapy was associated with decreased mortality compared with FQ monotherapy or FQ in combination with a BL in pneumococcal CAP and in patients with a higher systemic inflammatory response (CRP  $> 15$  mg/dL) [90]. When both factors were present, BM was protective against mortality on multivariate analysis [90]. No benefit was seen in those without a high inflammatory response and pneumococcal CAP, or in those with other

etiologies of CAP. In a more recent retrospective, observational study of patients with pneumococcal CAP (based on blood culture and urine antigen detection testing), patients with bacteremia were found to have a higher 30-day and 1-year all-cause mortality compared to those with negative blood cultures (26.2% versus 16.3%; age adjusted hazard ratio (aHR) 1.84; 95% CI 1.33-2.53 and 38.5% versus 30.4%; aHR 1.43; 95% CI 1.05-1.96, respectively) [91]. There was both a 30-day and 1-year mortality benefit in patients receiving combination therapy with a macrolide, but only for those patients with bacteremia, but not for those with negative blood cultures (mortality 18.8% versus 36.1%, aHR 0.49; 95% CI 0.30-0.80;  $p = 0.004$  and 31.3% versus 48.1%, respectively). In a prospective cohort study, BL therapy was compared with BM therapy (with use of azithromycin) in non-ICU cases hospitalized with CAP [92]. Severity of illness was assessed as mild-moderate and severe using the CURB-65 and Pneumonia Severity Index (PSI) scores and the Infectious Diseases Society of America (IDSA)/American Thoracic Society (ATS) criteria. Based on the CURB-65 and PSI scores, combination therapy did not significantly reduce 30-day mortality in either group. However, based on IDSA/ATS criteria, combination therapy significantly reduced 30-day mortality in patients with severe, but not non-severe, CAP (odds ratio (OR) 0.12; 95% CI 0.007-0.57 versus OR 1.85; 95% CI 0.51-5.40).

One single-center, retrospective cohort study in patients with severe CAP indicated that in those who had a negative BioFire PCR result, de-escalation from BM combination therapy was not associated with increased in-hospital mortality [93]. However, this fits in well with a more recent study of 2016 cases with bacteremic pneumococcal pneumonia, in whom 1921 were treated with empiric antibiotics with anti-pneumococcal coverage (ceftriaxone) and 1159 received “coverage for atypical pathogens” (macrolides or a fluoroquinolone) [73]. Independent predictors of mortality were age, being at high-risk for pneumococcal disease, and multilobar pneumonia, while female sex, and receipt of macrolide therapy were protective (OR 0.708; 95% CI 0.522-0.960 and OR 0.549; 95% CI 0.391-0.771, respectively). Treatment with either azithromycin or roxithromycin for as little as two days was protective and a full course of therapy was not required.

### *8.3. Conclusions Regarding the Clinical Benefits of Combination Therapy*

As indicated in the more recent studies described above, as well as in a review of the literature by Shumilak and colleagues [94], the bulk of the current evidence suggests that in adults hospitalized with CAP, BL monotherapy was equivalent to combination therapy in cases that were mild-to-moderate in severity, but that severe cases managed with BL monotherapy had worse clinical outcomes when compared with combination therapy. However, many of the authors of the publications described above, from which the conclusions were drawn, indicate that because of the limitation in quality and quantity of the studies, more randomized controlled trials with large numbers and high quality need to be performed to verify the findings [82,83,94–96].

Regarding additional mechanisms by which macrolides may attenuate cardiac events in severe CAP, it is interesting to note that in one pre-clinical trial, azithromycin and clarithromycin have been noted to possibly antagonize PAF [97]. One hypothesis-generating study was undertaken in patients with severe pneumonia (as defined by the IDSA/ATS guidelines) to determine if patients receiving a macrolide together with acetylsalicylic acid (ASA), in addition to conventional antibiotics would derive further benefit than those receiving conventional antibiotic therapy alone. Patients were divided into four groups: i) those receiving only ASA, ii) those receiving macrolides only, iii) those receiving ASA plus macrolide, and iv) those receiving neither ASA nor macrolide [98]. The mortality at 28-days was 15.5% in the ASA-macrolide group versus 28.2% in the group receiving neither agent and 23.8% in the macrolide group and 21.1% in the ASA group. After propensity score analysis, receipt of ASA plus a macrolide (HR 0.71; 95% CI 0.58-0.88;  $p=0.002$ ) was associated with a higher 30-day survival rate.

## **9. Are the Benefits of Combination Antibiotic Therapy Specifically due to the Macrolide?**

Two systematic reviews and meta-analyses compared the relative benefits of BM combination therapy with use of a BL plus FQ combination therapy (BLFQ) [95,96]. The authors indicated that one could not elicit strong conclusions on the findings because of high risk of bias, methodological issues,

and low quality of evidence in the various trials. The former study indicated that in the preliminary analysis, mortality of the BLFQ combination was higher than for BM combination therapy, but that in the meta-analysis with adjusted mortality rates there was no significant benefit of either of the two therapies (RR 1.26; 95% CI 0.95-1.67). However, the latter study in patients with severe CAP indicated that there was a significantly lower mortality in the BM versus the BLFQ therapy group and the length of hospital, but not ICU stay, was also lower. It is important to recognize that inter-class macrolide differences have been noted in some studies. While one systematic review and meta-analysis found no comparator studies between erythromycin and azithromycin, studies comparing erythromycin with clarithromycin showed erythromycin to be less effective [99]. Furthermore, a systematic review of azithromycin versus clarithromycin in combination with a BL, showed a higher success rate for azithromycin-based combinations, but a shorter hospital length of stay with clarithromycin-based combinations [100].

The reason for the differences in benefits of macrolide versus FQ combination therapy, if there are any, is not due to differences in efficacy against “atypical bacteria” as indicated in a recent systematic review and meta-analysis, which showed no difference in rates of clinical failure of CAP due to these pathogens [101]. It is also interesting that a recent retrospective study confirmed what other authors have previously shown, that the benefit of adding azithromycin to the antibiotic regimen in patients in ICU with pneumococcal CAP is significantly associated with a reduction in in-hospital mortality, independent of multiple co-variables even in the presence of macrolide resistance [102]. Furthermore, and rather interestingly, in a secondary analysis of the CAPO Internal Cohort study data, patients were grouped according to the sequence of administration of the macrolide and BL [103]. Overall, 99 patients were included in the “macrolide before” group and 305 in the “macrolide after” group. Administration of a macrolide before compared to after the BL reduced time to clinical stability (3 versus 4 days;  $p=0.011$ ), length of stay (6 versus 7 days;  $p=0.002$ ) and mortality 3% versus 7.2%;  $p=0.229$ ). Recent studies on the benefits of beta-lactam/macrolide combination therapy on outcome in adults hospitalized for CAP are summarized in Table 3.

**Table 3.** More recent studies on benefits of beta-lactam macrolide combination antibiotic therapy on outcome in adults hospitalized for CAP.

<u>Study, year</u>	<u>Country</u>	<u>Patient number</u>	<u>Type of study</u>	<u>Site of care</u>	<u>Comparison</u>	<u>Measure of severity of illness</u>	<u>Outcomes</u>
Ito et al. 2019	Japan	1131	Prospective cohort study. Hospitalized cases.	Non-ICU	BL versus BLM	PSI, CURB-65, IDSA/ATS criteria were measured. Patients were classified as mild, moderate, or severe.	Based on PSI and CURB-65 severity scores, combination therapy did not reduce 30-day mortality, in either treatment group. Based on IDSA/ATS criteria for severity, combination therapy significantly reduced 30-day mortality in severe but not non-severe pneumonia (OR 0.12; 95%CI 0.007-0.57).



Ceccato et al. 2019	Spain	1715	Prospective observational cohort study. Hospitalized cases of known microbial etiology.	ICU and non-ICU cases	BL plus FQ or FQ alone versus BLM	High inflammatory response (CRP > 15mg/dL)	BLM had a protective effect on mortality only in cases high inflammatory response and pneumococcal CAP (adjusted OR 0.28; 95% CI 0.09-0.93), but not in those without a high inflammatory response and pneumococcal CAP or with other etiologies.
Shorr et al 2021	USA	140	Retrospective cohort study of hospitalized patients with pneumococcal pneumonia, including cases with M-resistant pathogens	ICU	Comparison of outcomes in patients treated with antibiotic therapy with or without a M	Markers of acute and chronic disease (e.g., Charlson score, need for mechanical ventilation, and/or vasopressors and APACHE II)	The addition of M to the antibiotic regimen was associated with significant reduction in in-hospital mortality independent of multiple co-variables (adjusted odds ratio of death in those on macrolide 0.27; 95% CI 0.09-0.85; p = 0.024).
Goncalves-Pereira et al 2022	Portugal	797	Prospective multicenter study of hospitalized patients with pneumococcal CAP (bacteremic or non-bacteremic) with at least one comorbidity	ICU and non-ICU	Assessment of the benefit of BLM therapy versus non-BLM therapy in cases with and without bacteremia	-	Patients with bacteremia had higher 30-day all-cause mortality and BLM was beneficial only in patients with bacteremia (30-day all-cause mortality 18.9% versus 36.1%, aHR 0.49; 95% CI 0.30-0.80; p = 0.004). After 1-year follow-up patients with bacteremia who got BLM still had a lower all-cause mortality (31.3% versus 48.1%; p = 0.009).
Chowers et al. 2023	Israel	2016	Part of an ongoing prospective population	ICU and non-ICU cases	BLM versus other antibiotic therapy	Patients classified as no-risk, at-risk, and high-risk of	Macrolide therapy for as short as two days was protective against mortality (OR

-based active surveillance study adult cases with bacteremic pneumococcal pneumonia with no invasive macrolid pneumococcal disease. 0.549; 95% CI 0.391-0.771).

Abbreviations: ICU (intensive care unit); BL (beta-lactam); BLM (beta-lactam/macrolide combination FQ (fluoroquinolone); PSI (pneumonia severity index); CURB-65 (confusion, urea, respiratory rate, blood pressure, age≥65 years); OR (odds ratio); aHR (adjusted hazards ratio).

In addition to their antimicrobial activities, especially inhibitory effects on the production of Ply, it is noteworthy that macrolides specifically are associated with a myriad of non-antimicrobial actions that have been reviewed elsewhere [79].

10. Macrolide Antibiotics as Regulators of Pneumolysin Production

Many strategies, which target the reactivity of Ply have been described and represent the topic of several recent reviews [22,36,104]. These include a number of small molecules of natural and synthetic origin, monoclonal antibodies, liposome-encased cholesterol and vaccine-based strategies. Despite showing efficacy in murine models of experimental pneumococcal infection, these strategies, with the exception of cholesterol-containing liposomes [105], are, however, of unproven clinical efficacy. On a more positive note, innovative approaches to the development of pneumococcal protein-targeted vaccines show promise [106], as do competitive, synthetic peptides based on the Ply-binding site of the mannose receptor, MRC-1 [107].

Currently, however, inhibitors of Ply production, specifically macrolide antibiotics, appear to represent the most effective, inexpensive adjunctive option in augmenting the BL antibiotic-based treatment of IPD and presumably prevention of CVEs. In this context, the potential of macrolides, at sub-minimum inhibitory concentrations (MICs), to inhibit the production of Ply, not only by macrolide-susceptible, but also -resistant strains of the pathogen, has been known for over twenty years [108–111].

Macrolides are primarily inhibitors of bacterial protein synthesis, which bind to the peptide exit tunnel of the bacterial 50S ribosome, resulting in interference with peptide chain elongation [112]. Most commonly, macrolide resistance (≥256 µg/ml) results from acquisition by the pneumococcus of the inducible *erm(B)* gene, which encodes the enzyme ribosomal methylase. This enzyme dimethylates a single adenine residue in 23S ribosomal RNA, leading to high-level macrolide resistance. With respect to mechanisms by which macrolide-mediated attenuation of production of Ply is maintained in the setting of *erm(B)* positivity, Cockeran *et al.* reported that effective induction of *erm(B)* following exposure of strain 2507 of the pneumococcus (serotype 23F; MIC=≥ 256 µg/ml) to 0.5 µg/ml clarithromycin had a slow onset of approximately 12.5 hours [113]. This, in turn, resulted in transient susceptibility to clarithromycin and other macrolides, causing inhibition of both growth and production of Ply [113]. This mechanism was also detected in seven other *erm(B)*-expressing strains of the pneumococcus, albeit with variable lag times (4.9 – 11.5 hours) until full expression of the resistance phenotype. In addition, sequential exposure of strain 2507 to clarithromycin (0.5 µg/ml) followed 45 minutes later by ceftriaxone (0.05 – 0.5 µg/ml) resulted in significant, synergistic inhibition of bacterial growth [113].

More recently, Domon *et al.* described additional mechanisms of macrolide-mediated inhibition of Ply synthesis and release following exposure of strain NU4471 of the pneumococcus (serotype 19; MIC=≥1000 µg/ml) to azithromycin, clarithromycin or erythromycin (2 and 4 µg/ml) [114,115]. In

their initial study, focused on azithromycin and erythromycin, these authors reported that both macrolides, particularly erythromycin, caused inhibition of release of the autolysin, LytA, by the pneumococcus [114]. In addition, both macrolides caused significant down-regulation of transcription of the *ply* gene, while transcription of *lytA* was unaffected [114]. In a follow-up study, in which they compared clarithromycin with erythromycin, the dual mechanism of erythromycin-mediated inhibition of Ply synthesis and release was confirmed, while exposure to clarithromycin caused significant inhibition of transcription of the *ply* gene in the setting of potentiation of autolysis [115]. The findings of these two studies reported by Domon *et al.* appear to indicate that inhibition of transcription of the *ply* gene is a mechanism common to macrolide antibiotics, while effects on autolysis are variable. [114,115].

## 11. Conclusions

There remains a considerable global burden of community-acquired pneumonia, and in most areas of the world *S. pneumoniae* remains the predominant pathogen. It is now well-recognized that cardiovascular events are a relatively common occurrence in patients hospitalized with CAP and that the pneumococcal virulence factor, pneumolysin, plays a major direct role in these cardiac events, as well as having additional significant pro-inflammatory activities, which contribute, albeit indirectly, to these events. Regarding antibiotic therapy, it is generally accepted that the use of combined beta-lactam and macrolide therapy is associated with better outcomes in severely ill patients with CAP. In addition to their antibiotic effects, macrolides are well known to have anti-inflammatory, immunomodulatory activity, including significant ability to attenuate the production and activity of pneumolysin, even in the setting of macrolide resistance. Notwithstanding prior immunization of the vulnerable elderly against both the pneumococcus and influenza virus, adjunctive pneumolysin-targeted therapeutic strategies to support beta-lactam/macrolide combination therapy include the administration of cholesterol-encased liposomes and, albeit untested in the clinical setting, early administration of synthetic, diversionary MRC-1 peptide mimetics.

**Author Contributions:** Both authors contributed equally to conceptualization and compilation of the manuscript.

**Conflicts of Interest:** Neither author has a conflict of interest to declare.

## References

1. Ferreira-Coimbra, J.; Sarda, C.; Rello, J. Burden of community-acquired pneumonia and unmet clinical needs. *Adv. Ther.* **2020**, *37*, 1302-1318. doi: 10.1007/s12325-020-01248-7. PMID: 32072494.
2. Musher, D.M.; Abers, M.S.; Bartlett, J.G. Evolving understanding of the causes of pneumonia in adults, with special attention to the role of pneumococcus. *Clin. Infect. Dis.* **2017**, *65*, 1736-1744. doi: 10.1093/cid/cix549. PMID: 29028977.
3. Shoar, S.; Musher, D.M. Etiology of community-acquired pneumonia in adults: a systematic review. *Pneumonia* **2020**, *12*, 11. doi: 10.1186/s41479-020-00074-3. PMID: 33024653.
4. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* **2018**, *18*, 1191-1210. doi: 10.1016/S1473-3099(18)30310-4. PMID: 30243584.
5. Brueggemann, A.B.; Jansen van Rensburg, M.J.; Shaw, D.; McCarthy, N.D.; Jolley, K.A.; Maiden, M.C.J.; van der Linden, M.P.G.; Amin-Chowdhury, Z.; Bennett, D.E.; Borrow, R.; et al. Changes in the incidence of invasive disease due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data. *Lancet Digit. Health* **2021**, *3*, e360-e370. doi: 10.1016/S2589-7500(21)00077-7. PMID: 34045002.
6. Teng, J.L.L.; Fok, K.M.N.; Lin, K.P.K.; Chain, E.; Ma, Y.; Lau, S.K.P.; Woo, P.C.Y. Substantial decline in invasive pneumococcal disease during coronavirus disease 2019 pandemic in Hong Kong. *Clin. Infect. Dis.* **2022**, *74*, 335-338. doi: 10.1093/cid/ciab382. PMID: 33907808.
7. Perniciaro, S.; van der Linden, M.; Weinberger, D.M. Re-emergence of invasive pneumococcal disease in Germany during the spring and summer of 2021. *Clin. Infect. Dis.* **2022**, *75*, 1149-1153. doi: 10.1093/cid/ciac100. PMID: 35136983.

8. Corrales-Medina, V.F.; Musher, D.M.; Wells, G.A.; Chirinos, J.A.; Chen, L.; Fine, M.J. Cardiac complications in patients with community-acquired pneumonia incidence, timing, risk factors, and association with short-term mortality. *Circulation* **2012**, *125*, 773-781. doi: 10.1161/CIRCULATIONAHA.111.040766. PMID: 22219349.
9. Tralhão, A.; Póvoa, P. Cardiovascular events after community-acquired pneumonia: A global perspective with systematic review and meta-analysis of observational studies. *J. Clin. Med.* **2020**, *9*, 414. doi: 10.3390/jcm9020414. PMID: 32028660.
10. Pieralli, F.; Vannucchi, V.; Nozzoli, C.; Augello, G.; Dentali, F.; De Marzi, G.; Uomo, G.; Risaliti, F.; Morbidoni, L.; Mazzone, A.; et al; FADOI-ICECAP Study Group. Acute cardiovascular events in patients with community acquired pneumonia: results from the observational prospective FADOI-ICECAP study. *BMC Infect. Dis.* **2021**, *21*, 116. doi: 10.1186/s12879-021-05781-w. PMID: 33494707.
11. Musher, D.M.; Rueda, A.M.; Kaka, A.S.; Mapara, S.M. The association between pneumococcal pneumonia and acute cardiac events. *Clin. Infect. Dis.* **2007**, *45*, 158-165. doi: 10.1086/518849. PMID: 17578773.
12. Saikumar, H.; Anzueto, A.; Restrepo, M.I. Cardiovascular events and 1-year mortality in pneumococcal community-acquired pneumonia(CAP) compared to non-pneumococcal CAP. *Am. J. Respir. Crit. Care Med.* **2019**, *199*, A4230. <https://doi.org/10.1164/ajrccm-conference.2019.199.1.MeetingAbstracts.A4230>
13. Borsa, N.; Ruiz Iturriaga, L.A.; Serrano Fernandez, L.; España, P.P.; Aliberti, S.; Ramirez, J.A.; Sibila Vidal, O.S.; Rodrigo Troyano, A.M.; Rodriguez, A.H.; Sanz, F.; et al. Bacteremic pneumococcal pneumonia is associated with an increased rate of cardiovascular events. *Eur. Respir. J.* **2019**, *54*, OA3306. doi:10.1183/13993003.congress-2019.OA3306.
14. Rombauts, A.; Abelenda-Alonso, G.; Càmarà, J.; Lorenzo-Esteller, L.; González-Díaz, A.; Sastre-Escalà, E.; Gudíol, C.; Dorca, J.; Tebé, C.; Pallarès, N.; et al. Host- and pathogen-related factors for acute cardiac events in pneumococcal pneumonia. *Open Forum Infect. Dis.* **2020**, *7*, ofaa522. doi: 10.1093/ofid/ofaa522. PMID: 33335932.
15. Nishimura, N.; Fukuda, H. Risk of cardiovascular events leading to hospitalisation after *Streptococcus pneumoniae* infection: a retrospective cohort LIFE study. *BMJ Open* **2022**, *12*, e059713. doi:10.1136/bmjopen-2021-059713. PMID: 36332949.
16. Aldás, I.; Menéndez, R.; Méndez, R.; España, P.P.; Almirall, J.; Borderías, L.; Rajas, O.; Zalacaín, R.; Vendrell, M.; Mir, I.; et al; Grupo NEUMONAC. Early and late cardiovascular events in patients hospitalized for community-acquired pneumonia. *Arch. Bronconeumol.* **2020**, *56*, 551-558. doi: 10.1016/j.arbres.2019.10.009. PMID: 31791646.
17. Kruchow, K.L.; Zhao, K.; Bowdish, D.M.E.; Orihuela, C.J. Acute organ injury and long-term sequelae of severe pneumococcal infections. *Pneumonia* **2023**, *15*, 5. doi: 10.1186/s41479-023-00110-y. PMID: 36870980.
18. Africano, H.F.; Serrano-Mayorga, C.C.; Ramirez-Valbuena, P.C.; Bustos, I.G.; Bastidas, A.; Vargas, H.A.; Gómez, S.; Rodriguez, A.; Orihuela, C.J.; Reyes, L.F. Major adverse cardiovascular events during invasive pneumococcal disease are serotype dependent. *Clin. Infect. Dis.* **2021**, *72*, e711-e719. doi: 10.1093/cid/ciaa1427. PMID: 32964223.
19. Feldman, C.; Normark, S.; Henriques-Normark, B.; Anderson, R. Pathogenesis and prevention of risk of cardiovascular events in patients with pneumococcal community-acquired pneumonia. *J. Intern. Med.* **2019**, *285*, 635-652. doi: 10.1111/joim.12875. PMID: 30584680.
20. Fountoulaki, K.; Tsiordas, S.; Polyzogopoulou, E.; Olympios, C.; Parissis, J. Beneficial effects of vaccination on cardiovascular events: Myocardial infarction, stroke, heart failure. *Cardiology* **2018**, *141*, 98-106. doi: 10.1159/000493572. PMID: 30428464.
21. Jaiswal, V.; Ang, S.P.; Lnu, K.; Ishak, A.; Pokhrel, N.B.; Chia, J.E.; Hajra, A.; Biswas, M.; Matetic, A.; Dhatt, R.; et al. Effect of pneumococcal vaccine on mortality and cardiovascular outcomes: A systematic review and meta-analysis. *J. Clin. Med.* **2022**, *11*, 3799. doi: 10.3390/jcm11133799. PMID: 35807082.
22. Anderson, R.; Nel, J.G.; Feldman, C. Multifaceted role of pneumolysin in the pathogenesis of myocardial injury in community-acquired pneumonia. *Int. J. Mol. Sci.* **2018**, *19*, 1147. doi: 10.3390/ijms19041147. PMID: 29641429.
23. Mitsi, E.; Kamng'ona, R.; Rylance, J.; Solórzano, C.; Jesus Reiné, J.; Mwandumba, H.C.; Ferreira, D.M.; Jambo, K.C. Human alveolar macrophages predominately express combined classical M1 and M2 surface markers in steady state. *Respir. Res.* **2018**, *19*, 66. doi: 10.1186/s12931-018-0777-0. PMID: 29669565.
24. Martinez-Pomares, L. The mannose receptor. *J. Leukoc. Biol.* **2012**, *92*, 1177-1186. doi: 10.1189/jlb.0512231. PMID: 22966131.
25. Subramanian, K.; Neill, D.R.; Malak, H.A.; Spelmink, L.; Khandaker, S.; Dalla Libera Marchiori, G.; Dearing, E.; Kirby, A.; Yang, M.; Achour, A.; et al. Pneumolysin binds to the mannose receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival. *Nat. Microbiol.* **2019**, *4*, 62-70. doi: 10.1038/s41564-018-0280-x. PMID: 30420782.
26. Periselneris, J.; Turner, C.T.; Ercoli, G.; Szylar, G.; Weight, C.M.; Thurston, T.; Whelan, M.; Tomlinson, G.; Noursadeghi, M.; Brown, J. Pneumolysin suppresses the initial macrophage pro-inflammatory response to *Streptococcus pneumoniae*. *Immunology* **2022**, *167*, 413-427. doi: 10.1111/imm.13546. PMID: 35835695.



27. Inomata, M.; Xu, S.; Chandra, P.; Meydani, S.N.; Takemura, G.; Philips, J.A.; Leong, J.M. Macrophage LC3-associated phagocytosis is an immune defense against *Streptococcus pneumoniae* that diminishes with host aging. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 33561-33569. doi: 10.1073/pnas.2015368117. PMID: 33376222.
28. Ligeon, L.A.; Pena-Francesch, M.; Münz, C. Measuring oxidation within LC3-associated phagosomes that optimizes MHC class II restricted antigen presentation. *Methods Cell Biol.* **2021**, *164*, 187-200. doi: 10.1016/bs.mcb.2021.02.003. PMID: 34225915.
29. Cole, J.; Angyal, A.; Emes, R.D.; Mitchell, T.J.; Dickman, M.J.; Dockrell, D.H. Pneumolysin is responsible for differential gene expression and modifications in the epigenetic landscape of primary monocyte derived macrophages. *Front. Immunol.* **2021**, *12*, 573266. doi: 10.3389/fimmu.2021.573266. PMID: 34046027.
30. Paulikat, A.D.; Tölken, L.A.; Jachmann, L.H.; Burchhardt, G.; Hammerschmidt, S.; Siemens, N. *Streptococcus pneumoniae* impairs maturation of human dendritic cells and consequent activation of CD4+ T cells via pneumolysin. *J. Innate Immun.* **2022**, *14*, 569-580. doi: 10.1159/000522339. PMID: 35249041.
31. Price, K.E.; Camilli, A. Pneumolysin localizes to the cell wall of *Streptococcus pneumoniae*. *J. Bacteriol.* **2009**, *191*, 2163-2168. doi: 10.1128/JB.01489-08. PMID: 19168620.
32. Price, K.E.; Greene, N.G.; Camilli, A. Export requirements of pneumolysin in *Streptococcus pneumoniae*. *J. Bacteriol.* **2012**, *194*, 3651-3660. doi: 10.1128/JB.00114-12. PMID: 22563048.
33. Kadioglu, A.; Taylor, S.; Iannelli, F.; Pozzi, G.; Mitchell, T.J.; Andrew, P.W. Upper and lower respiratory tract infection by *Streptococcus pneumoniae* is affected by pneumolysin deficiency and differences in capsule type. *Infect. Immun.* **2002**, *70*, 2886-2890. doi: 10.1128/IAI.70.6.2886-2890.2002. PMID: 12010976.
34. Dhuriya, Y.K.; Sharma, D. Necroptosis: a regulated inflammatory mode of cell death. *J. Neuroinflammation* **2018**, *15*, 199. doi: 10.1186/s12974-018-1235-0. PMID: 29980212.
35. Riegler, A.N.; Brissac, T.; Gonzalez-Juarbe, N.; Orihuela, C.J. Necroptotic cell death promotes adaptive immunity against colonizing pneumococci. *Front. Immunol.* **2019**, *10*, 615. doi: 10.3389/fimmu.2019.00615. PMID: 31019504.
36. Nishimoto, A.T.; Rosch, J.W.; Tuomanen, E.I. Pneumolysin: Pathogenesis and therapeutic target. *Front. Microbiol.* **2020**, *11*, 1543. doi: 10.3389/fmicb.2020.01543. PMID: 32714314.
37. Witzernath, M.; Gutbier, B.; Hocke, A.C.; Schmeck, B.; Hippenstiel, S.; Berger, K.; Mitchell, T.J.; de los Toyos, J.R.; Rosseau, S.; Suttorp, N.; et al. Role of pneumolysin for the development of acute lung injury in pneumococcal pneumonia. *Crit. Care Med.* **2006**, *34*, 1947-1954. doi: 10.1097/01.CCM.0000220496.48295.A9. PMID: 16715037.
38. Rubins, J.B.; Charboneau, D.; Paton, J.C.; Mitchell, T.J.; Andrew, P.W.; Janoff, E.N. Dual function of pneumolysin in the early pathogenesis of murine pneumococcal pneumonia. *J. Clin. Invest.* **1995**, *95*, 142-150. doi: 10.1172/JCI117631. PMID: 7814608.
39. González-Juarbe, N.; Bradley, K.M.; Shenoy, A.T.; Gilley, R.P.; Reyes, L.F.; Hinojosa, C.A.; Restrepo, M.I.; Dube, P.H.; Bergman, M.A.; Orihuela, C.J. Pore-forming toxin-mediated ion dysregulation leads to death receptor-independent necroptosis of lung epithelial cells during bacterial pneumonia. *Cell Death Differ.* **2017**, *24*, 917-928. doi: 10.1038/cdd.2017.49. PMID: 28387756.
40. Cockeran, R.; Theron, A.J.; Steel, H.C.; Matlola, N.M.; Mitchell, T.J.; Feldman, C.; Anderson, R. Proinflammatory interactions of pneumolysin with human neutrophils. *J. Infect. Dis.* **2001**, *183*, 604-611. doi: 10.1086/318536. PMID: 11170986.
41. Witzernath, M.; Gutbier, B.; Owen, J.S.; Schmeck, B.; Mitchell, T.J.; Mayer, K.; Thomas, M.J.; Ishii, S.; Rosseau, S.; Suttorp, N.; et al. Role of platelet-activating factor in pneumolysin-induced acute lung injury. *Crit. Care Med.* **2007**, *35*, 1756-1762. doi: 10.1097/01.CCM.0000269212.84709.23. PMID: 17522574.
42. Dalal, P.J.; Muller, W.A.; Sullivan, D.P. Endothelial cell calcium signaling during barrier function and inflammation. *Am. J. Pathol.* **2020**, *190*, 535-542. doi: 10.1016/j.ajpath.2019.11.004. PMID: 31866349.
43. Gutbier, B.; Neuhaus, A.K.; Reppe, K.; Ehrler, C.; Santel, A.; Kaufmann, J.; Scholz, M.; Weissmann, N.; Morawietz, L.; Mitchell, T.J.; et al; CAPNETZ and PROGRESS Study Groups. Prognostic and pathogenic role of angiopoietin-1 and -2 in pneumonia. *Am. J. Respir. Crit. Care Med.* **2018**, *198*, 220-231. doi: 10.1164/rccm.201708-1733OC. PMID: 29447449.
44. Thurston, G.; Daly, C. The complex role of angiopoietin-2 in the angiopoietin-tie signaling pathway. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a006550. doi: 10.1101/cshperspect.a006550. PMID: 22951441.
45. Lefrançois, E.; Ortiz-Muñoz, G.; Caudrillier, A.; Mallavia, B.; Liu, F.; Sayah, D.M.; Thornton, E.E.; Headley, M.B.; David, T.; Coughlin, S.R.; et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* **2017**, *544*, 105-109. doi: 10.1038/nature21706. PMID: 28329764.
46. Lefrançois, E.; Looney, M.R. Platelet biogenesis in the lung circulation. *Physiology* **2019**, *34*, 392-401. doi: 10.1152/physiol.00017.2019. PMID: 31577166.
47. Nel, J.G.; Durandt, C.; Mitchell, T.J.; Feldman, C.; Anderson, R.; Tintinger, G.R. Pneumolysin mediates platelet activation *in vitro*. *Lung* **2016**, *194*, 589-593. doi: 10.1007/s00408-016-9900-5. PMID: 27192991.
48. Letsiou, E.; Teixeira Alves, L.G.; Felten, M.; Mitchell, T.J.; Müller-Redetzky, H.C.; Dudek, S.M.; Witzernath, M. Neutrophil-derived extracellular vesicles activate platelets after pneumolysin exposure. *Cells* **2021**, *10*, 3581. doi: 10.3390/cells10123581. PMID: 34944089.

49. Ohkuni, H.; Nagamune, H.; Ozaki, N.; Tabata, A.; Todome, Y.; Watanabe, Y.; Takahashi, H.; Ohkura, K.; Kourai, H.; Ohtsuka, H.; et al. Characterization of recombinant *Streptococcus mitis*-derived human platelet aggregation factor. *APMIS* **2012**, *120*, 56-71. doi: 10.1111/j.1600-0463.2011.02813.x. PMID: 22151309.
50. Jahn, K.; Handtke, S.; Palankar, R.; Weißmüller, S.; Nouailles, G.; Kohler, T.P.; Wesche, J.; Rohde, M.; Heinz, C.; Aschenbrenner, A.F.; et al. Pneumolysin induces platelet destruction, not platelet activation, which can be prevented by immunoglobulin preparations *in vitro*. *Blood Adv.* **2020**, *4*, 6315-6326. doi: 10.1182/bloodadvances.2020002372. PMID: 33351126.
51. Nel, J.G.; Durandt, C.; Theron, A.J.; Tintinger, G.R.; Pool, R.; Richards, G.A.; Mitchell, T.J.; Feldman, C.; Anderson, R. Pneumolysin mediates heterotypic aggregation of neutrophils and platelets *in vitro*. *J. Infect.* **2017**, *74*, 599-608. doi: 10.1016/j.jinf.2017.02.010. PMID: 28267572.
52. Nel, J.G.; Theron, A.J.; Durandt, C.; Tintinger, G.R.; Pool, R.; Mitchell, T.J.; Feldman, C.; Anderson, R. Pneumolysin activates neutrophil extracellular trap formation. *Clin. Exp. Immunol.* **2016**, *184*, 358-367. doi: 10.1111/cei.12766. PMID: 26749379.
53. McDonald, B.; Davis, R.P.; Kim, S.J.; Tse, M.; Esmon, C.T.; Kolaczowska, E.; Jenne, C.N. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood* **2017**, *129*, 1357-1367. doi: 10.1182/blood-2016-09-741298. PMID: 28073784.
54. Zucoloto, A.Z.; Jenne, C.N. Platelet-neutrophil interplay: Insights into neutrophil extracellular trap (NET)-driven coagulation in infection. *Front. Cardiovasc. Med.* **2019**, *6*, 85. doi: 10.3389/fcvm.2019.00085. PMID: 31281822.
55. Radermecker, C.; Detrembleur, N.; Guiot, J.; Cavalier, E.; Henket, M.; d'Emal, C.; Vanwinge, C.; Cataldo, D.; Oury, C.; Delvenne, P.; et al. Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. *J. Exp. Med.* **2020**, *217*, e20201012. doi: 10.1084/jem.20201012. PMID: 32926097.
56. Leppkes, M.; Knopf, J.; Naschberger, E.; Lindemann, A.; Singh, J.; Herrmann, I.; Stürzl, M.; Staats, L.; Mahajan, A.; Schauer, C.; et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. *EBioMedicine* **2020**, *58*, 102925. doi: 10.1016/j.ebiom.2020.102925. PMID: 32745993.
57. Moorthy, A.N.; Rai, P.; Jiao, H.; Wang, S.; Tan, K.B.; Qin, L.; Watanabe, H.; Zhang, Y.; Teluguakula, N.; Chow, V.T. Capsules of virulent pneumococcal serotypes enhance formation of neutrophil extracellular traps during *in vivo* pathogenesis of pneumonia. *Oncotarget* **2016**, *7*, 19327-19340. doi: 10.18632/oncotarget.8451. PMID: 27034012.
58. Wolfmeier, H.; Schoenauer, R.; Atanassoff, A.P.; Neill, D.R.; Kadioglu, A.; Draeger, A.; Babychuk, E.B. Ca<sup>2+</sup>-dependent repair of pneumolysin pores: A new paradigm for host cellular defense against bacterial pore-forming toxins. *Biochim. Biophys. Acta* **2015**, *1853*, 2045-2054. doi: 10.1016/j.bbamcr.2014.09.005. PMID: 25219550.
59. Larpin, Y.; Besançon, H.; Iacovache, M.I.; Babychuk, V.S.; Babychuk, E.B.; Zuber, B.; Draeger, A.; Köffel, R. Bacterial pore-forming toxin pneumolysin: Cell membrane structure and microvesicle shedding capacity determines differential survival of cell types. *FASEB J.* **2020**, *34*, 1665-1678. doi: 10.1096/fj.201901737RR. PMID: 31914676.
60. Nerlich, A.; von Wunsch Teruel, I.; Mieth, M.; Hönzke, K.; Rückert, J.C.; Mitchell, T.J.; Suttrop, N.; Hippenstiel, S.; Hocke, A.C. Reversion of pneumolysin-induced executioner caspase activation redirects cells to survival. *J. Infect. Dis.* **2021**, *223*, 1973-1983. doi: 10.1093/infdis/jiaa639. PMID: 33045080.
61. Batori, R.K.; Chen, F.; Bordan, Z.; Haigh, S.; Su, Y.; Verin, A.D.; Barman, S.A.; Stepp, D.W.; Chakraborty, T.; Lucas, R.; et al. Protective role of Cav-1 in pneumolysin-induced endothelial barrier dysfunction. *Front. Immunol.* **2022**, *13*, 945656. doi: 10.3389/fimmu.2022.945656. PMID: 35967431.
62. Wiese, A.D.; Mitchel, E.; Ndi, D.; Markus, T.M.; Talbot, H.K.; Schaffner, W.; Grijalva, C.G. The risk of acute myocardial infarction among patients with laboratory-confirmed invasive pneumococcal disease: a self-controlled case series study. *Clin. Infect. Dis.* **2023**, ciad065. doi: 10.1093/cid/ciad065. PMID: 36751004. Epub ahead of print.
63. Brown, A.O.; Mann, B.; Gao, G.; Hankins, J.S.; Humann, J.; Giardina, J.; Faverio, P.; Restrepo, M.I.; Halade, G.V.; Mortensen, E.M.; et al. *Streptococcus pneumoniae* translocates into the myocardium and forms unique microlesions that disrupt cardiac function. *PLoS Pathog.* **2014**, *10*, e1004383. doi: 10.1371/journal.ppat.1004383. PMID: 25232870.
64. Brown, A.O.; Millett, E.R.; Quint, J.K.; Orihuela, C.J. Cardiotoxicity during invasive pneumococcal disease. *Am. J. Respir. Crit. Care Med.* **2015**, *191*, 739-745. doi: 10.1164/rccm.201411-1951PP. PMID: 25629643.
65. Reyes, L.F.; Restrepo, M.I.; Hinojosa, C.A.; Soni, N.J.; Anzueto, A.; Babu, B.L.; Gonzalez-Juarbe, N.; Rodriguez, A.H.; Jimenez, A.; Chalmers, J.D.; et al. Severe pneumococcal pneumonia causes acute cardiac toxicity and subsequent cardiac remodeling. *Am. J. Respir. Crit. Care Med.* **2017**, *196*, 609-620. doi: 10.1164/rccm.201701-0104OC. PMID: 28614669.
66. Shenoy, A.T.; Beno, S.M.; Brissac, T.; Bell, J.W.; Novak, L.; Orihuela, C.J. Severity and properties of cardiac damage caused by *Streptococcus pneumoniae* are strain dependent. *PLoS One* **2018**, *13*, e0204032. doi: 10.1371/journal.pone.0204032. PMID: 30216364.

67. Stevens, E.J.; Morse, D.J.; Bonini, D.; Duggan, S.; Brignoli, T.; Recker, M.; Lees, J.A.; Croucher, N.J.; Bentley, S.; Wilson, D.J.; et al. Targeted control of pneumolysin production by a mobile genetic element in *Streptococcus pneumoniae*. *Microb. Genom.* **2022**, *8*, 000784. doi: 10.1099/mgen.0.000784. PMID: 35416147.
68. Gilley, R.P.; González-Juarbe, N.; Shenoy, A.T.; Reyes, L.F.; Dube, P.H.; Restrepo, M.I.; Orihuela, C.J. Infiltrated macrophages die of pneumolysin-mediated necroptosis following pneumococcal myocardial invasion. *Infect. Immun.* **2016**, *84*, 1457-1469. doi: 10.1128/IAI.00007-16. PMID: 26930705.
69. Shenoy, A.T.; Brissac, T.; Gilley, R.P.; Kumar, N.; Wang, Y.; Gonzalez-Juarbe, N.; Hinkle, W.S.; Daugherty, S.C.; Shetty, A.C.; Ott, S.; et al. *Streptococcus pneumoniae* in the heart subvert the host response through biofilm-mediated resident macrophage killing. *PLoS Pathog.* **2017**, *13*, e1006582. doi: 10.1371/journal.ppat.1006582. PMID: 28841717.
70. Beno, S.M.; Riegler, A.N.; Gilley, R.P.; Brissac, T.; Wang, Y.; Kruckow, K.L.; Jadapalli, J.K.; Wright, G.M.; Shenoy, A.T.; Stoner, S.N.; et al. Inhibition of necroptosis to prevent long-term cardiac damage during pneumococcal pneumonia and invasive disease. *J. Infect. Dis.* **2020**, *222*, 1882-1893. doi: 10.1093/infdis/jiaa295. PMID: 32492702.
71. Platt, M.P.; Lin, Y.H.; Wiscovitch-Russo, R.; Yu, Y.; Gonzalez-Juarbe, N. Pandemic influenza infection promotes *Streptococcus pneumoniae* Infiltration, necrotic damage, and proteomic remodeling in the heart. *MBio.* **2022**, *13*, e0325721. doi: 10.1128/mbio.03257-21. PMID: 35089061.
72. Syed, S.; Nissilä, E.; Ruhanen, H.; Fudo, S.; Gaytán, M.O.; Sihvo, S.P.; Lorey, M.B.; Metso, J.; Öörni, K.; King, S.J.; et al. *Streptococcus pneumoniae* pneumolysin and neuraminidase A convert high-density lipoproteins into pro-atherogenic particles. *IScience* **2021**, *24*, 102535. doi: 10.1016/j.isci.2021.102535. PMID: 34124613.
73. Chowers, M.; Gerassy-Vainberg, S.; Cohen-Poradosu, R.; Wiener-Well, Y.; Bishara, J.; Maor, Y.; Zimhony, O.; Chazan, B.; Gottesman, B.; Dagan, R.; et al.; IAIPD research group. The effect of macrolides on mortality in bacteremic pneumococcal pneumonia: A retrospective, Nationwide Cohort Study, Israel, 2009-2017. *Clin. Infect. Dis.* **2022**, *75*, 2219-2224. doi: 10.1093/cid/ciac317. PMID: 35443039.
74. Metlay, J.P.; Waterer, G.W.; Long, A.C.; Anzueto, A.; Brozek, J.; Crothers, K.; Cooley, L.A.; Dean, N.C.; Fine, M.J.; Flanders, S.A.; et al. Diagnosis and treatment of adults with community-acquired pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am. J. Respir. Crit. Care Med.* **2019**, *200*, e45-e67. doi: 10.1164/rccm.201908-1581ST. PMID: 31573350.
75. Gupta, V.; Yu, K.C.; Schranz, J.; Gelone, S.P. A multicenter evaluation of the US prevalence and regional variation in macrolide-resistant *S. pneumoniae* in ambulatory and hospitalized adult patients in the United States. *Open Forum Infect. Dis.* **2021**, *8*, ofab063. doi: 10.1093/ofid/ofab063. PMID: 34250183.
76. Niederman, M.S. Macrolide-resistant pneumococcus in community-acquired pneumonia. Is there still a role for macrolide therapy? *Am. J. Respir. Crit. Care Med.* **2015**, *191*, 1216-1217. doi: 10.1164/rccm.201504-0701ED. PMID: 26029831.
77. Waterer, G. Empiric antibiotics for community-acquired pneumonia: A macrolide and a beta-lactam please! *Respirology* **2018**, *23*, 450-451. doi:10.1111/resp.13248. PMID: 29278281.
78. Cilloniz, C.; Albert, R.K.; Liapikou, A.; Gabarrus, A.; Rangel, E.; Bello, S.; Marco, F.; Mensa, J.; Torres, A. The effect of macrolide resistance on the presentation and outcome of patients hospitalized for *Streptococcus pneumoniae* pneumonia. *Am. J. Respir. Crit. Care Med.* **2015**, *191*, 1265-1272. doi: 10.1164/rccm.201502-0212OC. PMID: 25807239.
79. Kricker, J.A.; Page, C.P.; Gardarsson, F.R.; Baldursson, O.; Gudjonsson, T.; Parnham, M.J. Nonantimicrobial actions of macrolides: Overview and perspectives for future development. *Pharmacol. Rev.* **2021**, *73*, 233-262. doi: 10.1124/pharmrev.121.000300. PMID: 34716226.
80. Metlay, J.P.; Waterer, G.W. Treatment of community-acquired pneumonia during the coronavirus disease 2019 (COVID-19) pandemic. *Ann. Intern. Med.* **2020**, *173*, 304-305. doi: 10.7326/M20-2189. PMID: 32379883.
81. Giamarellos-Bourboulis, E.J.; Daikos, G.L.; Gargalianos, P.; Gogos, C.; Lazanas, M.; Panagopoulos, P.; Poulakou, G.; Sambatakou, H.; Samarkos, M. The role of macrolides for the management of community-acquired pneumonia and pneumonia by the novel coronavirus SARS-CoV-2 (COVID-19): A position paper by four medical societies from Greece. *Infect. Dis. Ther.* **2021**, *10*, 1081-1095. doi: 10.1007/s40121-021-00471-1. PMID: 34137001.
82. Liu, S.; Tong, X.; Ma, Y.; Wang, D.; Huang, J.; Zhang, L.; Wu, M.; Wang, L.; Liu, T.; Fan, H. Respiratory fluoroquinolones monotherapy vs.  $\beta$ -lactams with or without macrolides for hospitalized community-acquired pneumonia patients: A meta-analysis. *Front. Pharmacol.* **2019**, *10*, 489. doi: 10.3389/fphar.2019.00489. PMID: 31139081.
83. Xu, L.Y.; Wang, C.C.; Peng, X.X.; Jiao, Y.; Zhao, C.Z.; Zhang, L.; Ma, L. Empirical antibiotic treatment strategies for community-acquired pneumonia: a network meta-analysis. *J. Glob. Antimicrob. Resist.* **2022**, *30*, 1-9. doi: 10.1016/j.jgar.2022.05.009. PMID: 35643393.
84. Gillbert, T.T.; Arfstrom, R.J.; Mihalovic, S.W.; Dababneh, A.S.; Varatharaj Palraj, B.R.; Dierkhising, R.A.; Mara, K.C.; Lessard, S.R. Effect of  $\beta$ -lactam plus macrolide versus fluoroquinolone on 30-day readmissions for community-acquired pneumonia. *Am. J. Ther.* **2020**, *27*, e177-e182. doi:10.1097/MJT.0000000000000788. PMID: 30418221.

85. Kim, Y.; Jeon, Y.; Kwon, K.T.; Bae, S.; Hwang, S.; Chang, H.H.; Kim, S.W.; Lee, W.K.; Yang, K.H.; Shin, J.H.; et al. Beta-lactam plus macrolide for patients hospitalized with community-acquired pneumonia: Difference between autumn and spring. *J. Korean Med. Sci.* **2022**, *37*, e324. doi: 10.3346/jkms.2022.37.e324. PMID: 36413797.
86. König, R.; Cao, X.; Oswald, M.; Forstner, C.; Rohde, G.; Rupp, J.; Witzernath, M.; Welte, T.; Kolditz, M.; Pletz, M.; CAPNETZ study group. Macrolide combination therapy for patients hospitalised with community-acquired pneumonia? An individualised approach supported by machine learning. *Eur. Respir. J.* **2019**, *54*, 1900824. doi: 10.1183/13993003.00824-2019. PMID: 31537702.
87. Zhang, Y.Q.; Zou, S.L.; Zhao, H.; Zhang, M.M.; Han, C.L. Ceftriaxone combination therapy versus respiratory fluoroquinolone monotherapy for community-acquired pneumonia: A meta-analysis. *Am. J. Emerg. Med.* **2018**, *36*, 1759-1765. doi: 10.1016/j.ajem.2018.01.079. PMID: 29499898.
88. Salunkhe, V.; Furmanek, S.P.; Arnold, F.W. Beta-lactam plus a macrolide versus a fluoroquinolone for empiric therapy of hospitalized patients with community-acquired pneumonia: Results from the University of Louisville Pneumonia Study. *University of Louisville Journal of Respiratory Infections (ULJRI)* **2019**, *3*, 6. doi: 10.18297/jri/vol3/iss1/6
89. Suzuki, J.; Sasabuchi, Y.; Hatakeyama, S.; Matsui, H.; Sasahara, T.; Morisawa, Y.; Yamada, T.; Fushimi, K.; Yasunaga, H. Additional effect of azithromycin over  $\beta$ -lactam alone for severe community-acquired pneumonia-associated acute respiratory distress syndrome: a retrospective cohort study. *Pneumonia* **2022**, *14*, 1. doi: 10.1186/s41479-021-00093-8. PMID: 35012674.
90. Ceccato, A.; Cilloniz, C.; Martin-Loeches, I.; Ranzani, O.T.; Gabarrus, A.; Bueno, L.; Garcia-Vidal, C.; Ferrer, M.; Niederman, M.S.; Torres, A. Effect of combined  $\beta$ -lactam/macrolide therapy on mortality according to the microbial etiology and inflammatory status of patients with community-acquired pneumonia. *Chest* **2019**, *155*, 795-804. doi: 10.1016/j.chest.2018.11.006. PMID: 30471269.
91. Gonçalves-Pereira, J.; Costa, L.; Silva, I.; Simões, A.; Froes, F.; Mergulhão, P.; Varela Ramos, P.; Leal, D.; Alves, R.; Custódio, M.; et al. The benefit of macrolide therapy in patients with pneumococcal pneumonia is only present in patients with bacteremia. *Pulmonology* **2022** Nov 1:S2531-0437(22)00205-7. doi: 10.1016/j.pulmoe.2022.08.003. PMID: 36333235. Epub ahead of print.
92. Ito, A.; Ishida, T.; Tachibana, H.; Tokumasu, H.; Yamazaki, A.; Washio, Y. Azithromycin combination therapy for community-acquired pneumonia: propensity score analysis. *Sci. Rep.* **2019**, *9*, 18406. doi: 10.1038/s41598-019-54922-4. PMID: 31804572.
93. Hopkins, T.M.; Juang, P.; Weaver, K.; Koffef, M.H.; Betthausen, K.D. Outcomes of macrolide de-escalation in severe community-acquired pneumonia. *Clin. Ther.* **2019**, *41*, 2540-2548. doi: 10.1016/j.clinthera.2019.10.005. PMID: 31676040.
94. Shumilak, G.; Sligl, W.I. Moving past the routine use of macrolides – reviewing the role of combination therapy in community-acquired pneumonia. *Curr. Infect. Dis. Rep.* **2018**, *20*, 45. doi: 10.1007/s11908-018-0651-8. PMID: 3019133.
95. Li, J.; Peng, Y.; Li, X. Meta-analysis of the effects of combination therapies of  $\beta$ -lactams and fluoroquinolones or macrolides in the treatment of community-acquired pneumonia. *Am. J. Transl. Res.* **2021**, *13*, 2439-2446. PMID: 34017402.
96. Lee, J.H.; Kim, H.J.; Kim, Y.H. Is  $\beta$ -lactam plus macrolide more effective than  $\beta$ -lactam plus fluoroquinolone among patients with severe community-acquired pneumonia?: a systemic review and meta-analysis. *J. Korean Med. Sci.* **2017**, *32*, 77-84. doi: 10.3346/jkms.2017.32.1.77. PMID: 27914135.
97. Tsoupras, A.B.; Chini, M.; Tsogas, N.; Lioni, A.; Tsekis, G.; Demopoulos, C.A.; Lazanas, M.C. *In vitro* anti-inflammatory and anti-coagulant effects of antibiotics towards Platelet Activating Factor and thrombin. *J. Inflamm.* **2011**, *8*, 17. doi: 10.1186/1476-9255-8-17. PMID: 21736752.
98. Falcone, M.; Russo, A.; Shindo, Y.; Farcomeni, A.; Pieralli, F.; Cangemi, R.; Lui, J.; Xia, J.; Okumura, J.; Sano, M.; et al. A hypothesis-generating study of the combination of aspirin plus macrolides in patients with severe community-acquired pneumonia. *Antimicrob. Agents Chemother.* **2019**, *63*, e01556-18. doi: 10.1128/AAC.01556-18. PMID: 30509940.
99. Ashy, N.; Alharbi, L.; Alkhamisi, R.; Alradadi, R.; Eljaaly, K. Efficacy of erythromycin compared to clarithromycin and azithromycin in adults or adolescents with community-acquired pneumonia: A systematic review and meta-analysis of randomized controlled trials. *J. Infect. Chemother.* **2022**, *28*, 1148-1152. doi: 10.1016/j.jiac.2022.04.020. PMID: 35523718.
100. Al-Salloum, J.; Gillani, S.W.; Mahmood, R.K.; Gulam, S.M. Comparative efficacy of azithromycin versus clarithromycin in combination with beta-lactams to treat community-acquired pneumonia in hospitalized patients: a systematic review. *J. Int. Med. Res.* **2021**, *49*, 3000605211049943. doi: 10.1177/03000605211049943. PMID: 34719987.
101. Basilim, A.; Wali, H.; Rabaan, A.A.; Eljaaly, K. Efficacy of empiric macrolides versus fluoroquinolones in community-acquired pneumonia associated with atypical bacteria: A meta-analysis. *Respir. Med. Res.* **2022**, *82*, 100931. doi: 10.1016/j.resmer.2022.100931. PMID: 35905552.



102. Shorr, A.F.; Simmons, J.; Hampton, N.; Micek, S.T.; Kollef, M.H. Pneumococcal community-acquired pneumonia in the intensive care unit: Azithromycin remains protective despite macrolide resistance. *Respir. Med.* **2021**, *177*, 106307. doi: 10.1016/j.rmed.2021.106307. PMID: 33486205.
103. Peyrani, P.; Wiemken, T.L.; Metersky, M.L.; Arnold, F.W.; Mattingly, W.A.; Feldman, C.; Cavallazzi, R.; Fernandez-Botran, R.; Bordon, J.; Ramirez, J.A. The order of administration of macrolides and beta-lactams may impact the outcomes of hospitalized patients with community-acquired pneumonia: results from the Community-Acquired Pneumonia Organization. *Infect. Dis.* **2018**, *50*, 13-20. doi: 10.1080/23744235.2017.1350881. PMID: 28699429.
104. Cima Cabal, M.D.; Molina, F.; López-Sánchez, J.I.; Pérez-Santín, E.; Del Mar García-Suárez, M. Pneumolysin as a target for new therapies against pneumococcal infections: A systematic review. *PLoS One* **2023**, *18*, e0282970. doi: 10.1371/journal.pone.0282970. PMID: 36947540.
105. Laterre, P.F.; Colin, G.; Dequin, P.F.; Dugernier, T.; Boulain, T.; Azeredo da Silveira, S.; Lajaunias, F.; Perez, A.; François, B. CAL02, a novel antitoxin liposomal agent, in severe pneumococcal pneumonia: a first-in-human, double-blind, placebo-controlled, randomised trial. *Lancet Infect. Dis.* **2019**, *19*, 620-630. doi: 10.1016/S1473-3099(18)30805-3. PMID: 31056427.
106. Musher, D.M.; Anderson, R.; Feldman, C. The remarkable history of pneumococcal vaccination: an ongoing challenge. *Pneumonia* **2022**, *14*, 5. doi: 10.1186/s41479-022-00097-y. PMID: 36153636.
107. Subramanian, K.; Iovino, F.; Tsikourkitoudi, V.; Merkl, P.; Ahmed, S.; Berry, S.B.; Aschtgen, M.S.; Svensson, M.; Bergman, P.; Sotiriou, G.A.; et al. Mannose receptor-derived peptides neutralize pore-forming toxins and reduce inflammation and development of pneumococcal disease. *EMBO Mol. Med.* **2020**, *12*, e12695. doi: 10.15252/emmm.202012695. PMID: 32985105.
108. Lagrou, K.; Peetermans, W.E.; Jorissen, M.; Verhaegen, J.; Van Damme, J.; Van Eldere, J. Subinhibitory concentrations of erythromycin reduce pneumococcal adherence to respiratory epithelial cells *in vitro*. *J. Antimicrob. Chemother.* **2000**, *46*, 717-723. doi: 10.1093/jac/46.5.717. PMID: 11062190.
109. Fukuda, Y.; Yanagihara, K.; Higashiyama, Y.; Miyazaki, Y.; Hirakata, Y.; Mukae, H.; Tomono, K.; Mizuta, Y.; Tsukamoto, K.; Kohno, S. Effects of macrolides on pneumolysin of macrolide-resistant *Streptococcus pneumoniae*. *Eur. Respir. J.* **2006**, *27*, 1020-1025. doi: 10.1183/09031936.06.00116805. PMID: 16455827.
110. Anderson, R.; Steel, H.C.; Cockeran, R.; Smith, A.M.; von Gottberg, A.; de Gouveia, L.; Brink, A.; Klugman, K.P.; Mitchell, T.J.; Feldman, C. Clarithromycin alone and in combination with ceftriaxone inhibits the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of *Streptococcus pneumoniae*. *J. Antimicrob. Chemother.* **2007**, *59*, 224-229. doi: 10.1093/jac/dkl479. PMID: 17218449.
111. Anderson, R.; Steel, H.C.; Cockeran, R.; von Gottberg, A.; de Gouveia, L.; Klugman, K.P.; Mitchell, T.J.; Feldman, C. Comparison of the effects of macrolides, amoxicillin, ceftriaxone, doxycycline, tobramycin and fluoroquinolones, on the production of pneumolysin by *Streptococcus pneumoniae* *in vitro*. *J. Antimicrob. Chemother.* **2007**, *60*, 1155-1158. doi: 10.1093/jac/dkm338. PMID: 17848373.
112. Zuckerman, J.M. Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. *Infect. Dis. Clin. North Am.* **2004**, *18*, 621-649, xi-. doi: 10.1016/j.idc.2004.04.010. PMID: 15308279.
113. Cockeran R, Steel HC, Wolter N, de Gouveia L, von Gottberg A, Klugman KP, Leanord AT, Inverarity DJ, Mitchell TJ, Feldman C, et al. Effects of clarithromycin at sub-minimum inhibitory concentrations on early *ermB* gene expression, metabolic activity and growth of an *erm(B)*-expressing macrolide-resistant strain of *Streptococcus pneumoniae*. *Open Journal of Respiratory Diseases* **2012**, *2*, 1-8. doi: 10.4236/ojrd.2012.21001.
114. Domon, H.; Maekawa, T.; Yonezawa, D.; Nagai, K.; Oda, M.; Yanagihara, K.; Terao, Y. Mechanism of macrolide-induced inhibition of pneumolysin release involves impairment of autolysin release in macrolide-resistant *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* **2018**, *62*, e00161-18. doi: 10.1128/AAC.00161-18. PMID: 30181369.
115. Domon, H.; Isono, T.; Hiyoshi, T.; Tamura, H.; Sasagawa, K.; Maekawa, T.; Hirayama, S.; Yanagihara, K.; Terao, Y. Clarithromycin inhibits pneumolysin production via downregulation of *ply* gene transcription despite autolysis activation. *Microbiol. Spectr.* **2021**, *9*, e0031821. doi: 10.1128/Spectrum.00318-21. PMID: 34468195.

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.