Pathogenesis and Prevention of Risk of Cardiovascular Events in Patients with Pneumococcal Community-acquired Pneumonia

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Abstract

It is now well recognized that cardiovascular events (CVE) occur quite commonly, both in the acute phase and in the long-term, in patients with community-acquired pneumonia (CAP). CVE have been noted in up to 30% of patients hospitalized with all-cause CAP. One systematic review and meta-analysis of hospitalized patients with all-cause CAP noted that the incidence rates for overall cardiac events were 17.7%, for incident heart failure were 14.1%, for acute coronary syndromes were 5.3% and for incident cardiac arrhythmias were 4.7%. In the case of pneumococcal CAP, almost 20% of patients studied had one or more of these cardiac events. Recent research has provided insights into the pathogenesis of the acute cardiac events occurring in pneumococcal infections. With respect to the former, key involvements of the major pneumococcal protein virulence factor, pneumolysin, are now well documented, whilst systemic platelet-driven neutrophil activation may also contribute. However, events involved in the pathogenesis of the long-term cardiovascular sequelae remain largely unexplored. Emerging evidence suggests that persistent antigenaemia may predispose to the development of a systemic pro-inflammatory/prothrombotic phenotype underpinning the risk of future cardiovascular events. The current manuscript briefly reviews the occurrence of cardiovascular events in patients with all-cause CAP, as well as in pneumococcal and influenza infections. It highlights the close interaction between influenza and pneumococcal pneumonia. It also includes a brief discussion of mechanisms of the acute cardiac events in CAP. However, the primary focus is on the prevalence, pathogenesis and prevention of the longer-term cardiac sequelae of severe pneumococcal disease, particularly in the context of persistent antigenaemia and associated inflammation.

Key words: Community-acquired pneumonia, cardiovascular events, persistent antigenemia, pneumococcus, pneumolysin, vaccination

Introduction

It is clear that community-acquired pneumonia (CAP) remains an extremely common infection throughout the world, which continues to be associated with substantial morbidity and mortality [1–4]. There are geographical differences in the microbial etiology of CAP and recent studies from the United States of America (USA) have suggested that respiratory viruses are the most common cause, followed by *S. pneumoniae* (pneumococcus) [5]. Nevertheless, the pneumococcus is still the cause of some 10-15% of inpatient causes of CAP in the USA [1]. In other regions of the world, such as Europe, the pneumococcus remains the most common cause of CAP, with a considerable burden of disease [6, 7].

It is now well recognized that cardiac complications occur commonly in patients with CAP, particularly among hospitalized cases, and include acute myocardial infarction (AMI), new or worsening arrhythmia and new or worsening heart failure; these complications are associated with both short-term and long-term mortality [1–3]. This review will describe various aspects of cardiac complications, particularly in the setting of pneumococcal CAP.

Occurrence of cardiovascular events in patients with community-acquired pneumonia

Cardiovascular events (CVEs) have been noted to occur in patients with CAP, including those with all-cause CAP, in pneumococcal infections and in those with viral infections, especially influenza infections.

All-cause CAP

In 1984, Spodick and colleagues noted that acute respiratory symptoms occurred frequently in patients with AMI, significantly more so than in controls ($p<0.02$) [8]. The authors suggested that further investigations were required to determine whether there was any pathogenic relationship between these infections, which they presumed were viral, and the onset of AMI. Subsequently, Seedat *et al.* documented the occurrence of significant electrographic changes in patients with community-CAP, in association with cardiac enzyme leaks (creatinine kinase [CK]), including the cardiac fraction [CK-MB]), which were associated with the severity of infection [9]. More recently, a number of other investigators have documented the occurrence of a variety of CVEs in patients with CAP [10–12]. These findings were in agreement with those of an earlier systematic review and meta-analysis of complications in patients with CAP, which had indicated that significant cardiac complications occurred in a considerable number of cases [13]. The latter authors indicated that further research was required to identify the mechanisms of this association, the impact on patient outcomes and which patients are at highest risk, as well as to design preventative and treatment strategies [13].

While stroke and deep venous thrombosis have been identified as complications in patients with CAP, most CVEs involve the heart and include new or worsening heart failure, new or worsening arrhythmia (especially atrial fibrillation) and AMI [14, 15]. These CVEs, which occur in as many as 30% of hospitalized patients, are more common among older patients, nursing home residents, those with pre-existing CVEs and in severe pneumonia, but may also occur in cases with no apparent underlying risk factor(s) [14–18]. Most of the cardiac events were documented within the first week of illness, and more than 50% were noted to occur within the first 24 hours [15]. In the systematic review of observational studies, mentioned above, the incidence rates for overall cardiac complications were 17.7% (confidence interval [CI] 13.9-22.2), for incident heart failure were 14.1% (CI 9.3-20.6), for acute coronary syndromes were 5.3% (CI 3.2-8.6) and for incident cardiac arrhythmias were 4.7% (CI 2.4-8.9) [13].

While the occurrence of these CVEs was found to be associated with short-term mortality [14, 15], more importantly perhaps, these events have been documented to be associated with poor long-term prognosis, as well as with raised long-term risk of cardiovascular disease (CVD) [19–25]. Cardiovascular risk following an episode of CAP has been found to be highest in the first year, or in the first few years, following hospitalization; however, the increased risk has been found to extend as far out as 10 years in those studies that have evaluated the risk over that period of time [21-24]. This has prompted a number of investigators to derive risk stratification rules for determining which patients with CAP are at risk of acute cardiac complications [26, 27].

CAP due to Streptococcus pneumoniae

Griffin et al. in a study investigating risk factors for CVEs in patients with CAP noted that these occurred more frequently when *Staphylococcus aureus* or *Klebsiella pneumoniae* were the infecting pathogens [17], while the one study undertaken to derive a risk stratification tool for cardiac events, noted a greater risk in patients with CAP due to *Streptococcus pneumoniae* [26]. A second study investigating the differential roles of laboratory-confirmed pathogens as causes of both AMI and stroke documented that *S. pneumoniae* and the influenza virus were the most important causes of CVEs [28]. AMI rates associated with *S. pneumoniae* and influenza virus were increased substantially in the week following infection, with the rates of stroke being similarly high. Although point estimates for both outcomes were also raised for other respiratory viruses, they only reached significance for day 4-7 estimates for stroke. Musher and colleagues were among the first to document acute cardiac events in association with pneumococcal pneumonia (AMI, arrhythmia and new or worsening heart failure), which occurred in almost 20% of their patients with CAP [29]. The major cardiac events documented in 170 consecutive hospitalized patients with pneumococcal CAP in that study are summarized in Table 1. Furthermore, Geng and colleagues documented a case of Takotsubo cardiomyopathy in association with sepsis due to *S. pneumoniae* [30], while Gandhi and colleagues documented transient cardiomyopathy with myocarditis in severe infection due to *S. pneumoniae* [31].

CAP due to influenza virus infection

Cardiac complications from influenza infection, such as myocarditis, are well documented; however, it is also apparent from a number of studies that there is also a potential association between influenza infections and AMI [28, 32–37]. While association rates for AMI and other CVEs, such as stroke, are strongest with the influenza virus, other viruses, including respiratory syncytial virus (RSV), human metapneumovirus, rhinovirus and adenovirus, have also been implicated in the occurrence of CVEs. The reason for emphasizing the role of influenza virus in this current review, is that the potentially sinister interaction between this virus in particular, as well as other respiratory viruses, with pneumococcal pneumonia has been regularly described [38, 39]. One study, which sought to confirm the association between invasive pneumococcal pneumonia (IPP) and respiratory viral infections, examined 11 influenza seasons between 1994 and 2005 in the US [40]. The authors noted that IPP was associated with influenza and RSV in five seasons, with the strength of the association being highest when strain H3N2 was the predominant influenza A strain.

Experimental investigations, sometimes supported by *in vitro* studies of post-mortem human specimens, have investigated the interactions between pandemic influenza, including both the 1918 influenza pandemic and the H1N1 2009 influenza pandemic, and pneumococcal co-infection, to determine the reason(s) why the interaction of pneumococcal infections with pandemic influenza is associated with such considerable morbidity and mortality [41, 42]. Shrestha and colleagues, using weekly incidence data, noted that there was evidence of a strong, but relatively short-lived, interaction between influenza infection and pneumococcal infection resulting in an

approximately 100-fold increase in susceptibility to pneumococcal pneumonia [43]. In this context, the order and timing of influenza and pneumococcal infection are important, with experimental animal studies indicating that influenza infection occurring approximately 7 days before pneumococcal infection is associated with particularly severe infection [44].

With respect to pathogenesis, a number of transcriptional changes occur in the pneumococcus *per se* following influenza A virus infection, which are associated with fever and cell damage and result in the transition from asymptomatic nasopharyngeal colonization in biofilm, to an active, invasive pathogen [45]**.** This is supported by substantial evidence, much of it derived from animal models of experimental infection, that influenza virus infection is associated with alterations in the host respiratory tract that predispose to adherence, invasion and induction of disease by the pneumococcus [46]. These result in increased adhesion of the pneumococcus to virus-activated respiratory epithelium, together with alterations in pulmonary innate and adaptive immune responses that result in impaired clearance of the bacteria, as well as a chronic inflammatory response lasting for up to 26 weeks due to persistent viral antigenemia, specifically RNA, in the lower airways [47–49]. Pneumococcal replication in the airways may be further enhanced by an increased availability of free sialic acids derived from cleavage of host mucin by viral neuraminidase [50]. Another mechanism contributing to influenza virus-mediated persistence and replication of the pneumococcus relates to viral infection of airway dendritic cells, resulting in upregulation of intracellular expression of Toll-like receptor 3 (TLR3), which senses double-stranded RNA. This, in turn, may exacerbate pro-inflammatory cytokine production, not only via interactions of TLR3

with viral RNA, but also with bacterial endosomal RNA present in bystander dendritic cells infected with pneumococci [51].

Taken together, these findings, which are summarized in Table 2, may explain why preceding influenza virus infection, as well as infections caused by other types of respiratory viruses, leads to an increased bacterial burden in the airways, thereby contributing to both pneumococcal transmission and severity of disease [52–56].

Table 2: Mechanisms by which influenza virus infection predisposes for secondary pneumococcal infection

Mechanism	Reference
• Promotes transition of the pneumococcus from a nasopharyngeal colonist to an invasive pathogen	45
• Interferes with the protective activity of the mucociliary clearance system, while generating sialylated nutrients, which enhance the proliferation of the pneumococcus in the airways	46, 50
• Promotes increased attachment to, and invasion of respiratory epithelium via neuraminidase- and pro-inflammatory-dependent mechanisms	46
• Inhibits the phagocytic activity of pulmonary macrophages due to excessive production of interferon-gamma	47, 48
• Sustains a chronic, potentially immunosuppressive pulmonary phenotype due to persistence of pro-inflammatory viral RNA in the lungs	49

Mechanisms of acute myocardial injury in pneumococcal community-acquired pneumonia

Much recent research, largely based on murine [57–60] and non-human primate [61, 62] models of experimental pneumococcal disease, has focused on the pathogenesis of CVEs during the early, acute phase of severe pneumococcal

infection. Such research, which has been the subject of several recent reviews [11, 63–65] is covered only briefly here as the primary focus of the current review is on the prevalence, pathogenesis and prevention of the longer term cardiac sequelae of severe pneumococcal disease, particularly in the context of persistent antigenemia and associated inflammation.

Currently, two putative mechanisms have been implicated in the pathogenesis of myocardial damage and dysfunction during the acute, bacteremic phase of severe pneumococcal disease. One of these, which is also the best characterized, involves the cardiotoxic actions of the pneumococcal cholesterol-binding, pore-forming cytolysin, pneumolysin (PLY), while the other involves pro-thrombotic mechanisms involving various pneumococcal virulence factors, including PLY. The existence of the former mechanism has been demonstrated in both murine and non-human primate models of severe pneumococcal disease, which have revealed that invasion of the myocardium by the pneumococcus is an essential, initial step in induction of myocardial damage. [57, 58, 60]. Myocardial invasion by the pneumococcus is dependent on the pneumococcal adhesins, choline-binding protein A (CbpA) and phosphorylcholine, which promote trans-endothelial passage of the pathogen via binding to endothelial laminin and platelet-activating factor receptors, respectively [57]. The pneumococcal pilus-1 associated adhesin RrgA has also been reported to interact with the endothelial receptors, platelet-endothelial cell adhesion molecule-1 (PECAM-1) and the polymeric immunoglobulin receptor, to promote trans-endothelial passage of the bacteria from the circulation into the brain [66, 67]. In pilus-1 expressing pneumococci RrgA might therefore also contribute to translocation of the pathogen from the circulation into the heart tissue.

Invasion of the myocardium by the pneumococcus has been reported to result in the formation of early microlesions, enabling the pathogen to undergo intracellular invasion, survival and replication in small vesicles, which was dependent on bacterial adhesins, as well as the cytotoxins, PLY and pneumococcus-derived hydrogen peroxide, acting in concert [57, 68]. This was followed by development of mature microlesions, facilitated by PLY-mediated neutralization of resident, sentinel macrophages, in which the pneumococcus transitioned to a more persistent intracellular, biofilm-forming phenotype associated with high-level production of PLY [59, 69]. In this setting, myocardial damage and dysfunction appeared to result from the sub-lethal and lethal effects of the toxin, also acting in concert with hydrogen peroxide, on cardiomyocytes [57, 58, 68].

Although myocardial damage can also be induced by the systemic administration of recombinant PLY [70], a recent study has demonstrated that in the setting of severe, experimental pneumococcal infection caused by nine different serotypes of the pathogen, induction of cardiac damage is dependent on a high degree of bacteremia and is also strain specific [71]. Importantly, the nine pneumococcal strains tested varied with respect to their levels of PLY expression, albeit in artificial culture medium *in vitro*, possibly consistent with serotype-related, differential involvement of the toxin in the mediation of myocardial damage [71] and/or the ability of the pneumococcus to tightly regulate PLY production according to environmental conditions [72].

The second, prothrombotic/pro-inflammatory mechanism of pneumococcus-mediated myocardial damage has been less well explored in comparison with that

involving the direct cardiotoxic actions of PLY. The existence of this mechanism, which has recently been reviewed extensively elsewhere [64, 65] is based predominantly on data derived from *in vitro* experiments, as well as limited data from experimental animal models and clinical studies of severe CAP. It proposes that pathogen-driven systemic activation of platelets and neutrophils predisposes to the development of intra-vascular thrombosis, which may contribute to the pathogenesis of cardiovascular dysfunction [63, 64]. In this context, it is proposed that various pneumococcal virulence factors, including PLY, initiate and sustain a chain of events involving platelet activation and homotypic aggregation, as well as formation of heterotypic neutrophil:platelet aggregates, leading to the induction of neutrophil extracellular trap (NET) formation, thereby posing the potential risk of intravascular coagulation, microvascular dysfunction and cardiac damage.

These events are summarized in Figure 1.

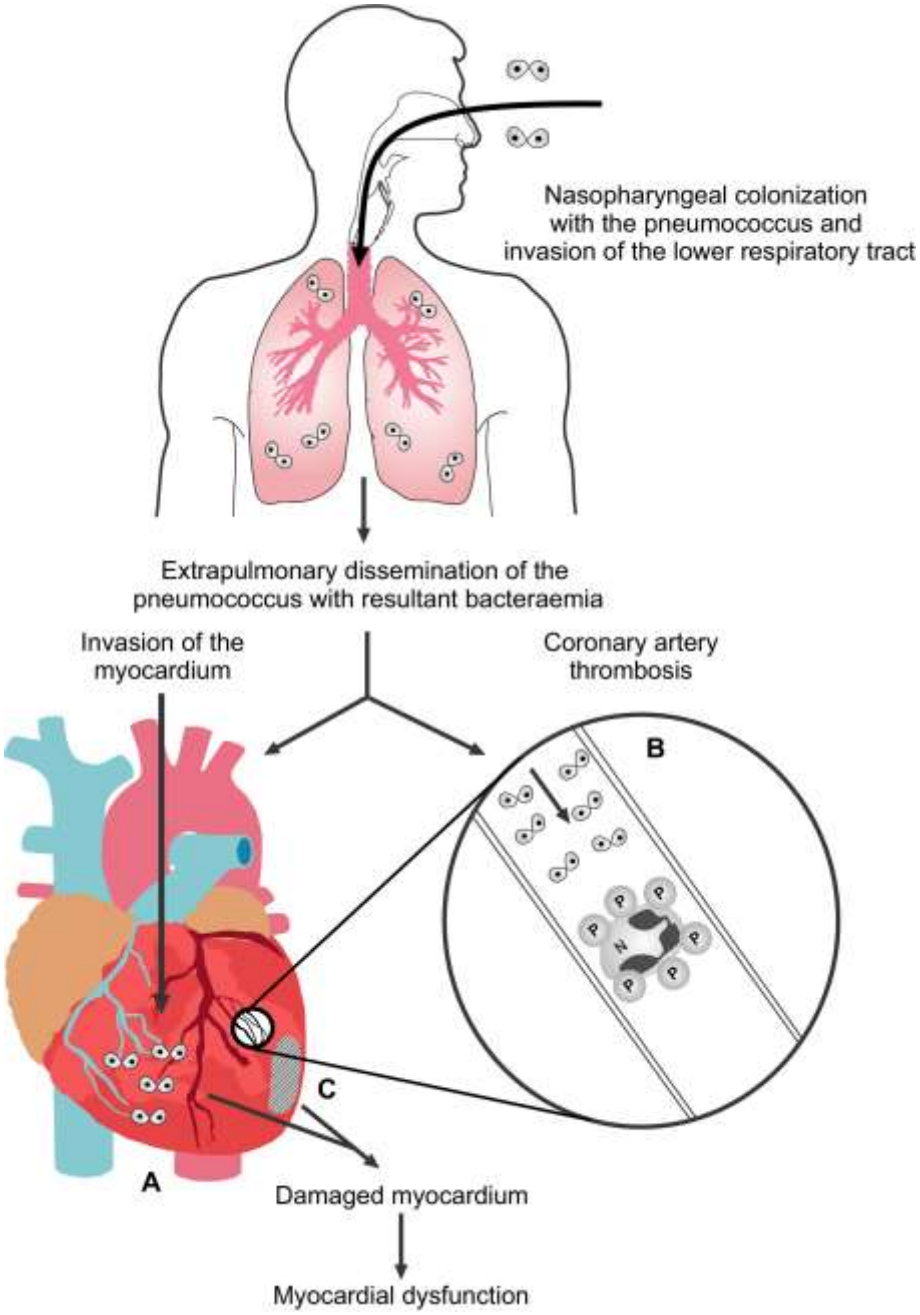

Figure 1. Proposed mechanisms involved in the pathogenesis of pneumolysin-mediated myocardial injury during invasive pneumococcal disease in humans. Following nasopharyngeal colonization, invasion of the lower respiratory tract, extra-pulmonary dissemination, and cardiac invasion by the pneumococcus (ΘΘ–symbol represents diplococci), intra-myocardial and intravascular release of pneumolysin (PLY) by the pathogen results in A: PLY-mediated death and dysfunction of cardiomyocytes; B: intravascular activation of platelets and neutrophils with resultant formation of pro-thrombotic/pro-NETotic neutrophil (N):platelet (P) aggregates (as illustrated in the magnification of an affected coronary arteriole/artery); and C: development of myocardial damage and dysfunction. Reproduced with permission of the International Journal of Molecular Sciences (Anderson R, Nel JG, Feldman C. Multifaceted Role of Pneumolysin in the Pathogenesis of Myocardial Injury in Community-Acquired Pneumonia. Int J Mol Sci 2018; 19: 1147.)

Persistent inflammation following clinical recovery from pneumococcal infections in the pathogenesis of CVE

There is an increasing awareness of the potential roles of persistent pneumococcal antigenemia and ongoing chronic inflammatory processes that follow clinical recovery from pneumococcal infections as interlinked drivers of long-term CVD, as well as infective and other adverse events.

Persistent antigenemia as a potential cause

Bacterial pathogens belonging to the genus *Streptococcus* are generally considered to be extracellular organisms. There are, however, some notable exceptions. For example, *S. pyogenes* has been found to survive intracellularly in human respiratory epithelial cells, neutrophils and macrophages [73–77]. In the case of macrophages, intracellular survival is dependent on the activity of the cholesterol-binding, pore-forming toxin, streptolysin-O, which promotes escape of the pathogen from phagolysosomes [75, 76]. This is achieved by toxin-mediated attenuation of vacuolar acidification, as well as induction of membrane damage, enabling escape and cytosolic growth of the pathogen [75, 76]. In this setting, the virulence of the pathogen is potentiated by the enzyme NAD-glycohydrolase (NADase), which depletes cellular energy stores [75].

Like its very close relative, the pneumococcus has also recently been reported to survive intracellularly in murine splenic $CD169^{+}$ (diphtheria toxin receptor)-expressing macrophages, a subset of these cells, which plays a key role in detecting and trapping blood-borne pathogens [78]. Intracellular survival of the pneumococcus in these splenic macrophages resulted in the establishment of a reservoir of the

pathogen, which, in turn, contributed to the development of fatal septicemia [78]. Interestingly, intracellular eradication of the pneumococcus was prevented by administration of macrolide antibiotics, which accumulate intracellularly, but not by beta-lactams [78].

In addition, it was recently published that PLY not only interacts with cholesterol, but also with the mannose receptor 1 (MRC-1), a receptor expressed by dendritic cells and M2-polarized macrophages [79]. MRC-1-PLY interaction enables pneumococci to enter cells in MRC-1- coated endosomes, a compartment which does not fuse with phagosomes. Consequently, pneumococci expressing cell surface-associated PLY can establish intracellular persistence in MRC-1-expressing immune cells, including dendritic cells and resident alveolar macrophages in the airways and possibly the myocardium. In this cellular compartment, PLY-expressing pneumococci mediate an anti-inflammatory response unlike the pro-inflammatory response typical of infected immune cells lacking MRC-1, such as M1-polarized macrophages, which may also favour intracellular persistence [79].

In the case of the heart, the exact cell type(s) implicated in intracellular replication and possible persistence of the pneumococcus remain to be identified, with cardiomyocytes, resident macrophages and/or fibroblasts being the most likely contenders.

Several studies involving human subjects infected with the pneumococcus are also consistent with the propensity of the pathogen, or its pro-inflammatory products, to persist beyond clinical recovery. A number of early studies, mainly involving patients

with pneumococcal pneumonia, in which capsular polysaccharide was detected in serum, sputum and/or urine using counter-current immune-electrophoresis and or latex agglutination procedures, described persistence of antigen for up to 3 weeks [80–83]. In another such study, pneumococcal capsular antigenemia, persisting for longer than 25 weeks after diagnosis, was detected in two of the nine patients tested [84]. Following the availability of an immunochromatographic procedure for the detection of pneumococcal capsular polysaccharide in urine, persistent antigenemia has been detected in 69.5% and 52.9% of patients at 1 month after hospital discharge and 1 month after diagnosis of pneumococcal pneumonia respectively [85, 86]. In the latter study, 66%, 33%, and 11% of urinary antigen-positive patients remained positive after 2, 4 and 6 months respectively [86].

The anatomical locations and cellular origins of reservoirs of pneumococcal polysaccharides following acute infection remain to be established. In this context, persistent antigenemia may reflect chronic release from resident tissue macrophages and other cell types of capsular polysaccharides derived from the remnants of previously eradicated pneumococci. Other possibilities include intracellular- and intra-biofilm-related persistence of the pneumococcus post-acute infection, as well as poor immunogenicity and variations in the rates of clearance of capsular polysaccharides from different serotypes of the pathogen. With respect to the latter scenario, the clearance rates of capsular polysaccharides of the pneumococcus derived from 12 different serotypes of the pneumococcus were found to vary by a factor of 250 in rabbits and rats [82]. Somewhat more speculatively, additional mechanisms include persistent antigenemia due to within-serotype mutations of genes encoding enzymes involved in the synthesis of capsular

polysaccharides, as has been reported to occur in variants of serotype 6 of the pneumococcus [87].

Irrespective of the mechanisms, which underpin the ongoing presence of pneumococcal capsular polysaccharides in the body fluids of some patients for several months following acute pneumococcal infection, it is probable that persistent antigenemia contributes to sustaining a pro-inflammatory/pro-coagulant phenotype. Important issues that remain to be resolved include the possible association of sustained antigenemia with specific capsular polysaccharide serotypes in humans, as well as the persistence of other types of pneumococcus-derived, pro-inflammatory structures, such as nucleic acid, cell-wall peptidoglycan and choline-binding proteins, membrane lipoproteins and various intracellular protein virulence factors, including PLY, concealed in pathogen-derived extracellular vesicles (EV) [88–91]. EVs are produced as protrusions from the bacterial plasma membrane and are readily taken up by immune cells. Unlike, encapsulated intact pneumococci, pneumococcal EVs contain exposed plasma membrane lipids resulting in serum complement deposition and activation of the complement cascade, a putative strategy by which the pneumococcus diverts and subverts host defenses [89].

The proposed mechanisms of ongoing antigenemia in the wake of acute pneumococcal infection are summarized in Figure 2.

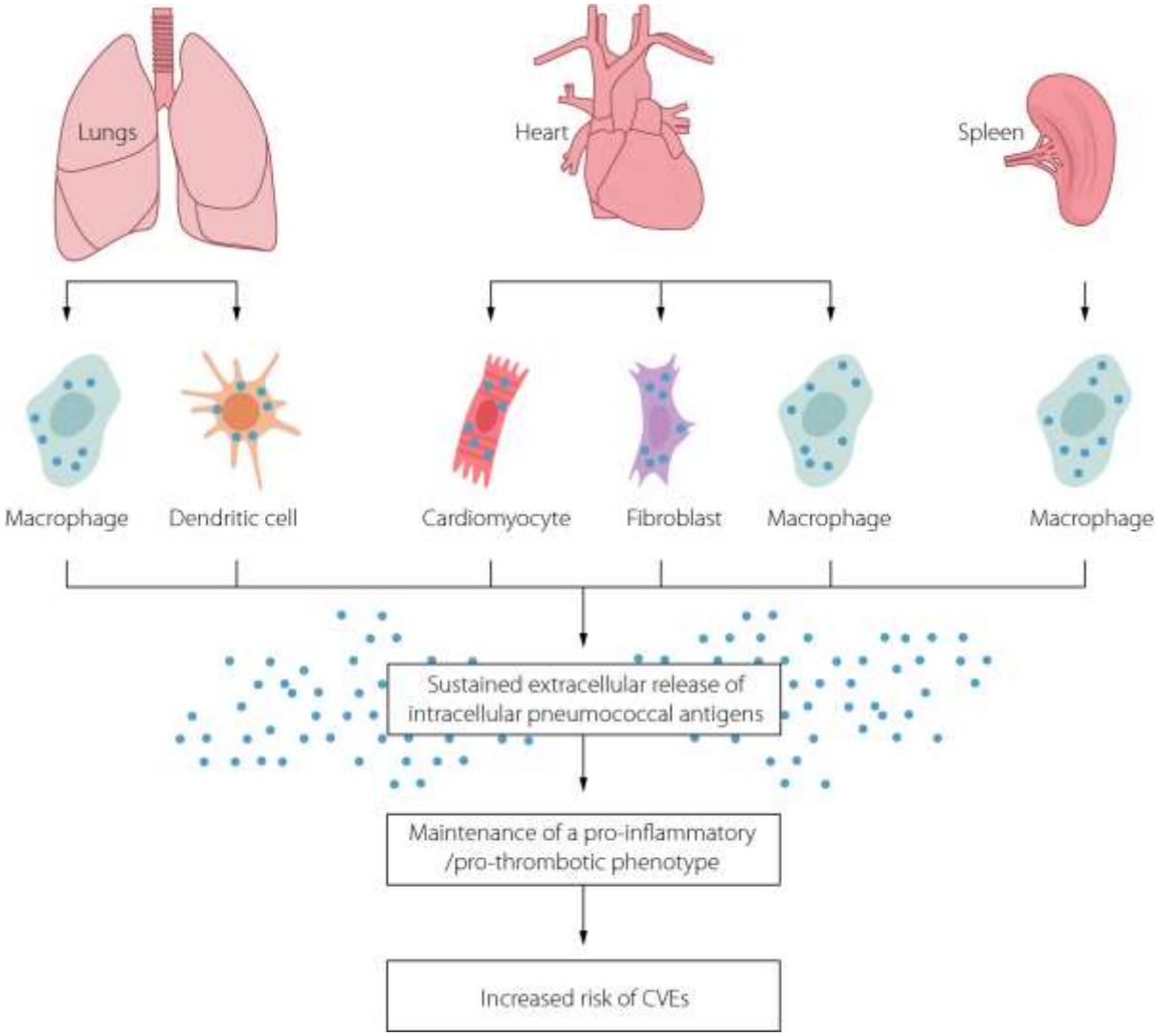

Figure 2. Proposed mechanisms of ongoing antigenemia (represented by black dots) in the post recovery phase of acute pneumococcal CAP and its relationship to sustained systemic pro-inflammatory/pro-thrombotic activity that may lead to delayed onset CVEs

Sustained inflammatory/procoagulant phenotype as a cause

Although there is considerable importance in understanding the relationship between *S. pneumoniae* infections and patient mortality, most previous studies have only used in-hospital or 30-day mortality as clinical end-points. One example was a cohort study in Sweden where logistic regression analyses were performed to assess risk factors for 30 days mortality of adult patients with community-acquired bacteremic pneumococcal pneumonia [92]. This study showed that

smoking, alcohol abuse, solid tumour, liver disease and renal disease were all significant attributors of the 30 days mortality, whereas heart disease was only associated with increased mortality in unadjusted, but not in adjusted analyses, suggesting confounding by other host factors, such as age, that was the most frequently documented risk factor.

More recent studies have investigated long-term mortality (arbitrarily defined as > 3 months), determining its frequency, risk factors, and implications for possible future interventions [93]. The risk of long-term mortality (up to 5 years and even longer) in patients who have survived an episode of pneumonia and its in-hospital related events is increased compared to that of cases hospitalized for other infections, as well as those hospitalized for other medical conditions. While older age and comorbid conditions may play a role in this increased mortality, some studies have suggested that this long-term mortality persists after adjusting for demographic characteristics (for example age), comorbidities and other risk factors [93]. Nevertheless, it is recognized that the risk factors for long-term mortality in patients with CAP include older age, male sex, race, type of pneumonia, chronic comorbid conditions, and severity of illness [93]. However, many of these conditions are also potential risk factors for sustained systemic inflammation after an episode of CAP.

An earlier study undertaken by Yende *et al.* reported an unusually high mortality rate in the 1–5 year period following discharge from hospital of older patients with CAP (n=3,075) [94]. Of the more than one-third of patients who succumbed within the 5-year period [94], mortality was most commonly associated with frequent re-hospitalization for a subsequent episode of pneumonia, CV and cerebrovascular

disease, as well as exacerbations of chronic obstructive pulmonary disease [94]. More recently, in another study also focused on hospitalized, older patients (n=1284), who had recovered from an episode of CAP caused predominantly by the pneumococcus, Adamuz *et al.* documented a mortality rate of 7.2% in the first year following hospital discharge [95]. The major causes of mortality in this study were infectious disease (48.4%), mostly pneumonia (25.8%), followed by acute CVEs (20.4%). Most deaths due to infectious disease (86.1%) occurred within the first 6 months, while mortality due to CV causes was stable throughout the 1-year period [95].

The authors of both of the aforementioned studies attributed the high level of susceptibility for development of repeat infections following the initial episode of CAP to residual, post-infective immunosuppression, while a pro-coagulant/pro-thrombotic phenotype resulting from persistent, low-grade, systemic inflammation was proposed to be a probable cause of CV dysfunction [94, 95]. The former contention is supported by several studies in patients with severe sepsis and CAP that have documented associations of both in-hospital and post-discharge mortality with elevated circulating levels of the anti-inflammatory/immunosuppressive cytokine, IL-10 [96–99].

Of these studies, one investigated the systemic cytokine response in patients with CAP and determined if there was a relationship between patterns of cytokine responses and severe sepsis and death [97]. Systemic cytokine levels were highest in CAP patients with fatal severe sepsis and lowest in patients without sepsis, with highest risk of death being in those with high levels of both the pro-inflammatory

cytokine interleukin-6 (IL-6) and the anti-inflammatory cytokine IL-10 activity (hazard ratio (HR) 20.5; 95% CI 10.8-39.0; p<0.001). Importantly, the duration of increased cytokine activity was longer than the clinical manifestations, and these cytokines remained elevated beyond clinical resolution. A separate study confirmed that systemic, but not bronchoalveolar, levels of IL-6 and IL-10, as well as interferon-gamma (INF-ɣ), were significantly higher in patients with severe CAP compared with non-severe CAP, with a good correlation with the Pneumonia Severity Index (PSI) [100]. In the whole group of patients with CAP (both severely ill and non-severely ill cases), IL-8 and IL-22 were increased in comparison to healthy controls and while IL-6 levels and IL-10 levels normalized after 7 days, IL-22 levels normalized only after 30 days, while IL-8 levels remained elevated after 30 days compared with healthy controls.

Furthermore, Yende and colleagues documented that persistent inflammation, defined as elevated systemic levels of IL-6 and IL-10, occurred at hospital discharge of patients with CAP and were associated with all-cause and cause-specific mortality over one year, despite there having been resolution of the clinical signs of acute infection [98]. The associations were not related to demographics, comorbidities or severity of infection. Of the 300 patients in whom the cause of death was identified, 30% was due to CVD. High IL-6 concentrations were associated with death due to CVD specifically, as well as cancer, infection and renal failure (p<0.008). In a different study, the same investigators studied survivors of CAP hospitalization from 28 US sites and noted that in 893 subjects, most of whom did not have severe disease, 88.4% had normal vital signs on hospital discharge, having apparently recovered from the acute infection [101]. However, D-dimer and thrombin-anti-

thrombin complex (TAT) levels were elevated in 78.8% and 30.1% of all the patients (51.3% and 25.3%, respectively, in those without severe sepsis). At one year post-hospital discharge, these raised levels were associated with a higher risk of all-cause mortality (HRs 1.66-1.17; p=0.0001 and 1.46-1.04; p=0.001 after adjusting for demographic characteristics and comorbidities) and CV mortality (p=0.009 and p=0.003, respectively, in competing risk analysis).

In an additional observational cohort study undertaken by the same investigators, which was focused on the impact of age, CAP patients were divided into five age groups, from < 50 years to $\geq$ 85 years [102]. Circulating inflammatory [tumor necrosis factor (TNF), IL-6 and IL-10)] biomarkers, as well as those of hemostasis (d-dimer, factor IX, thrombin-anti-thrombin complex, anti-thrombin and plasminogen-activator inhibitor-1) and cell surface activation [toll-like receptor (TLR)-2, TLR-4 and HLA-DR] were measured during the first week of hospitalization, and at discharge, and compared to 90 day mortality [102]. Significant differences were noted in the 90-day mortality in the different age groups (progressively higher in the progressively older age groups), which persisted at one year after discharge from hospital. There were no age-related differences in inflammatory or cell surface markers in the different age groups within the first week of hospitalization, but there were very modest increases in pro-coagulant markers in the elderly compared to the younger patients, which did not explain the large differences in mortality in the different age groups. On hospital discharge, and despite clinical resolution, with the presence of normal vital signs in >85% of patients, older patients had a modest increase in IL-6 levels and in hemostasis markers, suggesting a delayed resolution of the immune response compared to younger patients.

Lastly, since it has been recognized that there is a lower life expectancy in men, the same investigators undertook a prospective observational study of 2183 patients with CAP to assess whether the differences in survival of men versus women were due to clinical characteristics, the quality of care, or differences in the immune response [103]. At emergency department (ED) admission, men were noted to have significantly higher inflammatory [TNF, IL-6, IL-10), fibrinolysis (d-dimer) and lower coagulation (antithrombin III and factor IX)] biomarker levels ($p<0.05$). Survival in men was lower at 30, 90 and 365 days, and the one year mortality did not appear to be related to factors such as demographics, smoking, chronic comorbidities, clinical characteristics, severity of illness or vaccination status, but rather to differences in the levels of TNF, IL-6, IL-10, d-dimer, anti-thrombin III and factor X levels measured in the ED.

Rajas and colleagues, who noted that studies have indicated that the late prognosis of CAP is frequently related to CVEs, and that persistence of inflammatory markers is associated with higher long-term mortality from CAP, have published a protocol outlining a proposed study designed to investigate this relationship further [104]. In this prospective cohort study, various inflammatory markers will be measured in patients with CAP on admission and prior to discharge and the primary purpose will be to determine the ability of these inflammatory markers to predict both short-term and long-term prognosis in patients with CAP and to evaluate their relationship with CVEs.

Host- and pathogen-related factors, which may contribute to the maintenance of a systemic inflammatory phenotype following an episode of severe pneumococcal CAP, are documented in Table 3.

Table 3: Host- and pathogen-related factors which may contribute to the maintenance of a systemic inflammatory phenotype following an episode of severe pneumococcal CAP

Host-related	Pathogen-related
Predominantly factors associated with a low-grade, pre-existing, systemic pro-inflammatory/pro-thrombotic state, which is worsened and perpetuated by an episode of severe CAP [92-103]:* - Older age - Pre-existing CVD - Other co-morbidities - Male gender - Smoking/alcohol - Possible prior episode of influenza - Severity of illness	Predominantly factors related to immune evasion and intracellular persistence of the pneumococcus and its pro-inflammatory/pro-thrombotic products: - Immunogenicity of the capsular serotype of the pneumococcus, magnitude of bacteremia and impact on rate of clearance of the pathogen [82]; - Propensity to produce pneumolysin and intra-myocardial biofilm, enabling persistence in cardiomyocytes, cardiac fibroblasts and macrophages [59, 68, 69]; - Propensity to survive intracellularly in $CD169^{+}$ splenic macrophages, M2-polarized macrophages and dendritic cells [78, 79]; - Persistence of pneumococcal antigens in pathogen-derived extracellular vesicles [88-91]; - Persistence due to capsular switching [87].

*Relevant references indicated in parenthesis

Biomarkers of myocardial damage

In addition to commonly used biomarkers of cardiac injury such as cardiac troponins, creatine kinase, B-type natriuretic peptide and mid-regional pro-adrenomedullin,

Zemmour *et al.* have very recently described a novel, systemic biomarker of cardiomyocyte death *viz*. a cardiomyocyte-selective, unmethylated fragment of cell-free DNA [105]. This novel biomarker of cardiomyocyte death was found to be significantly elevated not only in patients with acute ST-elevation MI, but also in those with sepsis/septic shock, “suggesting a major role of cardiomyocyte death in mortality from sepsis” [105]. In this context there has also been a revival of interest in the clinical utility of measurement of circulating soluble triggering receptor expressed on myeloid cells (sTREM-1), described in an earlier study as being a useful pro-inflammatory biomarker in the diagnosis and prognosis, including mortality, in patients with severe all-cause CAP [106]. A more recent study has identified sTREM-1 as being a contributor to the pathogenesis of acute and chronic CV conditions, including AMI, with the authors contending that measurement of this biomarker has significant implications for diagnosis, management and prognosis of CV disorders [107].

Given that the pneumococcal serotype and extent of bacteremia appear to be determinants of myocardial invasion, albeit in the setting of experimental disease [71], measurement of the magnitude of bacterial DNAemia, reported to be a putative marker of disease severity [108], may also identify those at highest risk of development of acute and even late-onset CVEs.

Pneumococcal and influenza vaccination and the prevention of cardiovascular events

Since there is a significant volume of literature describing the occurrence of cardiac events in patients with CAP, including both all-cause and pneumococcal CAP, and since influenza is also known to predispose to pneumococcal infection, further

discussion of the potential benefit of both the pneumococcal and influenza vaccines, alone and in combination, in preventing CVEs is warranted.

Pneumococcal vaccination

There have been several studies [109–113], and some reviews [114], including systematic reviews and meta-analyses [115, 116] describing the potential benefits of the pneumococcal vaccine in preventing CVEs. Most of the studies have been in relationship to the use of the pneumococcal polyvalent polysaccharide vaccine (PPV23) and there are conflicting results. One hospital-based, case-control study, including patients in the study group that were considered to be at risk for AMI, noted that pneumococcal vaccine was associated with a >50% decrease in the rate of AMI, but the benefit was restricted to greater than two years following vaccination, which is difficult to understand [109]. Furthermore, in a prospective cohort study of men aged $\geq$ 45 years, who had no history of either AMI or stroke, with propensity score adjustment there was no evidence for a reduced risk of either AMI or stroke following pneumococcal vaccination [111]. The CAPAMIS study, which aimed, among other end-points, to assess the efficacy of PPV23 in reducing AMI and stroke in adults > 60 years, found efficacy of the vaccine against ischemic stroke, but no evidence of a reduced risk of AMI [112]. Eurich and colleagues documented that receipt of PPV23, even after adjusting for clinical data, was associated with a 60% reduction in acute coronary syndromes (ACS) among patients with pneumonia [113]. However, sensitivity analysis indicated that these results were probably the result of confounding, at least in part, most likely due to the so-called “healthy vaccine effect”. The authors further indicated that many of the studies in the literature rely on

databases and have important prognostic information lacking, such as severity of illness.

Two systematic reviews and meta-analyses attest to the benefit of PPV23 in preventing CVEs [115, 116]. Ren and colleagues noted in their meta-analysis of observational studies that use of PPV23 was associated with a significantly lower rate of ACS in the older population, but not of stroke [115]. The authors indicated that an adequately powered randomized controlled trial was essential to confirm these findings. The second meta-analysis, similarly, indicated that use of PPV23 was associated with a reduced risk of CVEs and death, with the beneficial effect increasing with age and in high cardiac risk patients and decreasing with time [116]. Ciszewski reviewed the data from recent studies and meta-analyses regarding the cardioprotective effects of the pneumococcal and influenza vaccines in patients with cardiovascular disease [114]. While the author noted that there was evidence of potential benefit of both vaccines in this high-risk group, he also indicated that in this age of evidence based medicine there was a lack of prospective, randomized controlled trials (RCT). In this context, it is noteworthy that Ren and colleagues [117] have recently registered the first RCT of PPV23 for prevention of CVEs.

Data on the efficacy of the pneumococcal conjugate vaccine, PCV13, in the prevention of CVEs await further studies. The landmark CAPiTA study demonstrated that PCV13 had a preventive effect on vaccine-type (VT) pneumococcal CAP and VT-type invasive pneumococcal disease (IPD) among adults 65 years of age or older [118], but did not provide information on prevention of CVEs in pneumococcal CAP. However, PCV13 is currently included in the national childhood vaccine programs of

many countries, which has led to a dramatic replacement of VT serotypes with non-VT serotypes among PCV-vaccinated children. Due to herd protection, colonization with non-VT strains has also subsequently occurred among adults, with these serotypes now accounting for the majority (60%) of cases of IPD among the elderly in countries such as Sweden [119, 120]. Furthermore, the overall incidence of IPD in this age group has not changed significantly in the post-PCV era.

According to the findings of a mouse model of experimental pneumococcal infection, it is only those pneumococcal serotypes capable of high-grade bacteremia that cause cardiac damage [71]. In this context, it is also well-recognized that the risk of bacteremia, as well as the risk of death from pneumococcal pneumonia or IPD, is serotype specific [121–123]. Thus the comparative ability of non-vaccine serotypes to cause high-grade bacteremia, compared to that of PCV13 serotypes, would determine the likely impact of PCV 13 on the occurrence of cardiac injury. Interestingly, clinical data derived from 2096 adults prior to PCV-introduction showed that patients infected with non-PCV13 strains had more underlying diseases, were less likely to have pneumonia and, tended to have a higher mortality in comparison to patients infected with PCV13 strains [124]. However, the invasive disease potential of these non-PCV13 serotypes was less than that of VT serotypes and, therefore, possibly less likely to be associated with CVEs.

Given these considerations, the use of PCV vaccination of older adults as a strategy to prevent CAP-associated CVEs is likely to be beneficial, but difficult to predict. Notwithstanding these considerations, the current recommendation by the Center for Disease Control and Prevention, USA for immunoprophylaxis of pneumococcal

infections in the elderly (≥ 65 years) is the so-called “prime boost strategy” [125], which consists of the administration of PCV13 followed, one year later, by PPV23, which would increase the potential cover of pneumococcal serotypes beyond that of PCV13 serotypes alone.

Future vaccines in development include those which are designed to confer broad, serotype-independent protection [126, 127]. Many of these are currently undergoing advanced evaluation for safety and efficacy, and include recombinant pneumococcal protein vaccines, including those targeting PLY, as well as non-encapsulated whole-cell vaccines.

Influenza vaccination

Similarly, for influenza vaccination there have been a number of studies [128–136], and some reviews [137, 138], including systematic reviews and meta-analyses [139] investigating its potential efficacy in preventing acute cardiac events. Influenza vaccination has been reported to reduce; i) the development of a new AMI in patients with chronic heart disease [128]; ii) the incidence of AMI in the 60 days following vaccination, compared to the baseline period [130]; and iii) the frequency of coronary ischemic events in patients with optimally treated coronary artery disease [129]. In a further study, influenza vaccine was significantly protective against AMI with a vaccine efficacy of 45% (95% CI 15% to 65%) [131]. A further two studies documented benefit of influenza vaccine against CVEs [132, 133]. Importantly, several of the authors indicated that despite the positive findings in their study, appropriate prospective studies were required.

In contrast, a number of studies have documented negative results. One indicated that there was no evidence of a reduced risk of AMI following receipt of the influenza vaccine [134]. A second reported that the benefit of influenza vaccine in elderly patients did not extend to prevention of recurrent coronary events [135], and a third noted that there was no reduction in major vascular events, including non-fatal AMI in patients following influenza vaccination, after consideration of all baseline characteristics [136].

While the review articles generally indicated benefit of influenza vaccination in reducing the incidence of AMI, one did indicate that vaccination rates still remained low [137, 138]. The authors of a systematic review and meta-analysis concluded that in patients with CVD, influenza vaccination appeared to reduce composite CVEs compared to placebo, but not individual outcomes such as AMI, and there was not enough evidence to determine whether vaccination was effective in primary prevention [139]. However, the authors also indicated that since most studies had some risk of bias, further higher quality evidence was required to confirm the findings. Fröbert and colleagues published the outline of a planned randomized trial to evaluate effectiveness of in-hospital influenza vaccination on death and cardiovascular outcomes in patients with ST-segment elevation myocardial infarction (STEMI) or non-STEMI [140].

Combined pneumococcal and influenza vaccination

A number of studies, including systematic reviews and meta-analyses, have indicated that there is an additive protective effect of combined pneumococcal and influenza vaccination on various end-points, such as occurrence of pneumonia,

hospitalization and death, and that dual vaccination is cost-effective [141–143]. However, there appears to be only one study investigating the effectiveness of combined pneumococcal and influenza vaccination in preventing AMI and stroke in the elderly with chronic illness, which demonstrated benefit against respiratory, as well as CV and cerebrovascular events, associated with reduced hospitalization, intensive care admission and death [110]. Interestingly, while one study documented an increased risk of AMI and stroke after a systemic respiratory tract infection, there was no evidence for the occurrence of these events in the period following the actual influenza or pneumococcal vaccination [144].

Conclusions

The association of severe all-cause CAP, especially pneumococcal CAP, with acute and delayed-onset CVEs is well recognized. Recent research, largely based on animal models of severe pneumococcal disease, has been revealing, most notably in providing insights into the pathogenesis of CVEs associated with acute disease. In this setting, myocardial invasion by the pathogen together with PLY-mediated cardiomyocyte dysfunction and cytotoxicity are key events, possibly exacerbated by the propensity of the pathogen to induce a pro-thrombotic state. Although the mechanisms underlying the pathogenesis of the delayed-onset CV sequelae of pneumococcal CAP are less well characterized, they appear to be related to a persistent, post-hospital discharge, systemic pro-inflammatory/pro-thrombotic phenotype. This may involve persistent antigenemia emanating from intracellular reservoirs of the pneumococcus and its pro-inflammatory remnants. Notwithstanding the effective implementation of pre-emptive anti-inflammatory and cardioprotective strategies, the identification of host- and/or pathogen-derived biomarkers with

accurate, predictive potential in relation to both acute and delayed-onset CVEs remains a priority. With respect to immunoprophylaxis of these CVEs, dual influenza/pneumococcal “prime boost” vaccination is promising, albeit largely untested, and may be rendered more efficacious through recognition of those serotypes of the pneumococcus with greatest predilection for invasion of the myocardium.

References

1. Musher DM, Thorner AR. Community-acquired pneumonia. *N Engl J Med* 2014; **371:** 1619 – 28.
2. Prina E, Ranzani OT, Torres A. Community-acquired pneumonia. *Lancet* 2015; **386:** 1097 – 108.
3. Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in adults. *BMJ* 2017; **358:** j2471.
4. Ramirez JA, Wiemken TL, Peyrani P, *et al*. Adults hospitalized with pneumonia in the United States: Incidence, Epidemiology, and mortality. *Clin Infect Dis* 2017; **65:** 1806 – 12.
5. Jain S, Self WH, Wunderink RG, *et al*. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med* 2015; **373:** 415 – 27.
6. Torres A, Blasi F, Peetermans WE, Viegi G, Welte T. The aetiology and antibiotic management of community-acquired pneumonia in adults in Europe: a literature review. *Eur J Clin Microbiol Infect Dis* 2014; **33:** 1065 – 79.
7. Torres A, Cillóniz C, Blasi F, *et al*. Burden of pneumococcal community-acquired pneumonia in adults across Europe: A literature review. *Respir Med* 2018; **137:** 6 – 13.

8. Spodick DH, Flessas AP, Johnson MM. Association of acute respiratory symptoms with onset of acute myocardial infarction: prospective investigation of 150 consecutive patients and matched control patients. *Am J Cardiol* 1984; **53:** 481 – 2.
9. Seedat MA, Feldman C, Skoularigis J, Promnitz DA, Smith C, Zwi S. A study of acute community-acquired pneumonia, including details of cardiac changes. *Q J Med* 1993; **86:** 669 – 75.
10. Corrales-Medina VF, Musher DM, Shachkina S, Chirinos JA. Acute pneumonia and the cardiovascular system. *Lancet* 2013; **381:** 496 – 505.
11. Feldman C, Anderson R. Prevalence, pathogenesis, therapy, and prevention of cardiovascular events in patients with community-acquired pneumonia. *Pneumonia (Nathan)* 2016; **8:** 11.
12. Restrepo MI, Reyes LF. Pneumonia as a cardiovascular disease. *Respirology* 2018; **23:** 250–9.
13. Corrales-Medina VF, Suh KN, Rose G, *et al*. Cardiac complications in patients with community-acquired pneumonia: A systematic review and meta-analysis of observational studies. *PLoS Medicine* 2011; **8:** e1001048.
14. Violi F, Cangemi R, Falcone M, *et al*. Cardiovascular complications and short-term mortality risk in community-acquired pneumonia. *Clin Infect Dis* 2017; **64:** 1486 – 93.
15. Corrales-Medina VF, Musher DM, Wells GA, Chirinos JA, Chen L, Fine MJ. Cardiac complications in patients with community-acquired pneumonia incidence, timing, risk factors, and association with short-term mortality. *Circulation* 2012; **125:** 773 – 81.

16. Ramirez J, Aliberti S, Mirsaeidi M, *et al*. Acute myocardial infarction in hospitalized patients with community-acquired pneumonia. *Clin Infect Dis* 2008; **47:** 182 – 7.
17. Griffin AT, Wiemken TL, Arnold FW. Risk factors for cardiovascular events in hospitalized patients with community-acquired pneumonia. *Int J Infect Dis* 2013; **17:** e1125 – 9.
18. Cilli A, Cakin O, Aksoy E, *et al*. Acute cardiac events in severe community-acquired pneumonia: A multicenter study. *Clin Respir J* 2018; **12:** 2212 – 9
19. Cangemi R, Calvieri C, Falcone M, *et al*. Relation of cardiac complications in the early phase of community-acquired pneumonia to long-term mortality and cardiovascular events. *Am J Cardiol* 2015; **116:** 647 – 51.
20. Corrales-Medina VF, Taljaard M, Yende S, *et al*. Intermediate and long-term risk of new-onset heart failure after hospitalization for pneumonia in elderly adults. *Am Heart J* 2015; **170:** 306 – 12.
21. Eurich DT, Marrie TJ, Minhas-Sandhu JK, Majumdar SR. Ten-year mortality after community-acquired pneumonia. A prospective cohort. *Am J Respir Crit Care Med* 2015; **192:** 597 – 604.
22. Corrales-Medina VF, Alvarez KN, Weissfeld LA, *et al*. Association between hospitalization for pneumonia and subsequent risk of cardiovascular disease. *JAMA* 2015; **313:** 264 – 7.
23. Bergh C, Fall K, Udumyan R, Sjöqvist H, Fröbert O, Montgomery S. Severe infections and subsequent delayed cardiovascular disease. *Eur J Prev Cardiol* 2017; **24**: 1958 – 66.

24. Eurich DT, Marrie TJ, Minhas-Sandhu JK, Majumdar SR. Risk of heart failure after community acquired pneumonia: prospective controlled study with 10 years of follow-up. *BMJ* 2017; **356:** j413.
25. Ruane L, Buckley T, Hoo SYS, *et al*. Triggering of acute myocardial infarction by respiratory infection. *Intern Med J* 2017; **47**: 522 – 9.
26. Viasus D, Garcia-Vidal C, Manresa F, Dorca J, Gudiol F, Carratalà J. Risk stratification and prognosis of acute cardiac events in hospitalized adults with community-acquired pneumonia. *J Infect* 2013; **66:** 27 – 33.
27. Corrales-Medina VF, Taljaard M, Fine MJ, *et al*. Risk stratification for cardiac complications in patients hospitalized for community-acquired pneumonia. *Mayo Clin Proc* 2014; **89:** 60 – 8.
28. Warren-Gash C, Blackburn R, Whitaker H, McMenamin J, Hayward AC. Laboratory-confirmed respiratory infections as triggers for acute myocardial infarction and stroke: a self-controlled case series analysis of national linked datasets from Scotland. *Eur Respir J* 2018; **51:** 101794.
29. Musher DM, Rueda AM, Kaka AS, Mapara SM. The association between pneumococcal pneumonia and acute cardiac events. *Clin Infect Dis* 2007; **45:** 158 – 65.
30. Geng S, Mullany D, Fraser JF. Takotsubo cardiomyopathy associated with sepsis due to *Streptococcus pneumoniae* pneumonia. *Crit Care Resusc* 2008; **10:** 231 – 4.
31. Gandhi T, Wu AH, Aronoff DM. Severe transient cardiomyopathy associated with community-acquired pneumonia caused by *Streptococcus pneumoniae*. *Heart Lung* 2018; **37:** 394 – 7.

32. Madjid M, Miller CC, Zarubaev VV, *et al*. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34 892 subjects. *Eur Heart J* 2007; **28**: 1205-10.
33. Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. *Lancet Infect Dis* 2009; **9:** 601-10.
34. Estabragh ZR, Mamas MA. The cardiovascular manifestations of influenza: A systematic review. *Int J Cardiol* 2013; **167:** 2397 – 403.
35. Barnes M, Heywood AE, Mahimbo A, Rahman B, Newall AT, Macintyre CR. Acute myocardial infarction and influenza: a meta-analysis of case–control studies. *Heart* 2015; **101:** 1738 – 47.
36. Kwong JC, Schwartz KL, Campitelli MA, *et al*. Acute myocardial infarction after laboratory-confirmed influenza infection. *N Engl J Med* 2018; **378:** 345 – 53.
37. Blackburn R, Zhao H, Pebody R, Hayward A, Warren-Gash C. Laboratory-confirmed respiratory infections as predictors of hospital admission for myocardial infarction and stroke: time-series analysis of English data for 2004 – 2015. *Clin Infect Dis* 2018; **67:** 8 – 17.
38. Martin-Loeches I, van Someren Gréve F, Schultz MJ. Bacterial pneumonia as an influenza complication. *Curr Opin Infect Dis* 2017; **30:** 201 – 7.
39. Rudd JM, Ashar HK, Chow VTK, Teluguakula N. Lethal synergism between influenza and *Streptococcus pneumoniae*. *J Infect Pulm Dis* 2016; **2**: doi: 10.16966/2470-3176.114.

40. Zhou H, Haber M, Ray S, Farley MM, Panozzo CA, Klugman KP. Invasive pneumococcal pneumonia and respiratory virus co-infections. *Emerg Infect Dis* 2012; **18:** 294 – 7.
41. Kash JC, Walters KA, Davis AS, *et al*. Lethal synergism of 2009 pandemic H1N1 influenza virus and *Streptococcus pneumoniae* coinfection is associated with loss of murine lung repair responses. *MBio* 2011; **2:** e00172-11.
42. Walters KA, D'Agnillo F, Sheng ZM, *et al*. 1918 pandemic influenza virus and *Streptococcus pneumoniae* co-infection results in activation of coagulation and widespread pulmonary thrombosis in mice and humans. *J Pathol* 2016; **238:** 85 – 97.
43. Shrestha S, Foxman B, Weinberger DM, Steiner C, Viboud C, Rohani P. Identifying the interaction between influenza and pneumococcal pneumonia using incidence data. *Sci Transl Med* 2013; **5:** 191ra84.
44. Davis BM, Aiello AE, Dawid S, Rohani P, Shrestha S, Foxman B. Influenza and community-acquired pneumonia interactions: The impact of order and time of infection on population patterns. *Am J Epidemiol* 2012; **175:** 363 – 7.
45. Pettigrew MM, Marks LR, Kong Y, Gent JF, Roche-Hakansson H, Hakansson AP. Dynamic changes in the *Streptococcus pneumoniae* transcriptome during transition from biofilm formation to invasive disease upon influenza A virus infection. *Infect Immun* 2014; **82:** 4607 – 19.
46. McCullers JA. Insight into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev* 2006; **19:** 571 – 82.
47. Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. *Nat Med* 2008; **14:** 558 – 64.

48. Dorrington MG, Roche AM, Chauvin SE, *et al.* MARCO is required for TLR2- and Nod2-mediated responses to *Streptococcus pneumoniae* and clearance of pneumococcal colonization in the murine nasopharynx. *J Immunol* 2013; **190:** 250 – 8.
49. Keeler SP, Agapov EV, Hinojosa ME, Letvin AN, Wu K, Holtzman MJ. Influenza A virus infection causes chronic lung disease linked to sites of active viral RNA remnants. *J Immunol* 2018; doi: 10.4049/jimmunol.1800671. [Epub ahead of print].
50. Siegel SJ, Roche AM, Weiser JN. Influenza promotes pneumococcal growth during coinfection by providing host sialylated substrates as a nutrient source. *Cell Host Microbe* 2014; **16:** 55 – 67.
51. Spelmink L, Sender V, Hentrich K, Kuri T, Plant L, Henriques-Normark B. Toll-like receptor 3/TRIF-dependent il-12p70 secretion mediated by *Streptococcus pneumoniae* RNA and its priming by Influenza A virus coinfection in human dendritic cells. *MBio* 2016; **7:** e00168-16.
52. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. *J Infect Dis* 2010; **202:** 1287 – 95.
53. Short KR, Habets MN, Hermans PWM, Diavatopoulos DA. Interactions between *Streptococcus pneumoniae* and influenza virus: a mutually beneficial relationship? *Future Microbiol* 2012; **7:** 609 – 24.
54. Lee KH, Gordon A, Foxman B. The role of respiratory viruses in the etiology of bacterial pneumonia: An ecological perspective. *Evol Med Public Health* 2016; **2016:** 95-109.

55. Karppinen S, Terajarvi J, Auranen K, *et al*. Acquisition and transmission of *Streptococcus pneumoniae* are facilitated during rhinovirus infection in families with children. *Am J Respir Crit Care Med* 2017; **196:** 1172 – 80.
56. Li Y, Peterson ME, Campbell H, Nair H. Association of seasonal viral acute respiratory infection with pneumococcal disease: a systematic review of population-based studies. *BMJ Open* 2018; **8:** e019743.
57. Brown AO, Mann B, Gao G, *et al*. *Streptococcus pneumoniae* translocates into the myocardium and forms unique microlesions that disrupt cardiac function. *PLoS Pathog* 2014; **10:** e1004383.
58. Brown AO, Millett ERC, Quint JK, Orihuela CJ. Cardiotoxicity during invasive pneumococcal disease. *Am J Respir Crit Care Med* 2015; **191:** 739 – 45.
59. Gilley RP, González-Juarbe N, Shenoy AT, *et al*. Infiltrated macrophages die of pneumolysin-mediated necroptosis following pneumococcal myocardial invasion. *Infect Immun* 2016; **84:** 1457 – 69.
60. Reyes LF, Restrepo MI, Hinojosa CA, *et al*. Severe pneumococcal pneumonia causes acute cardiac toxicity and subsequent cardiac remodeling. *Am J Respir Crit Care Med* 2017; **196:** 609 – 20.
61. Kraft BD, Piantadosi CA, Benjamin AM, *et al*. Development of a novel preclinical model of pneumococcal pneumonia in nonhuman primates. *Am J Respir Cell Mol Biol* 2014; **50:** 995 – 1004.
62. Reyes LF, Restrepo MI, Hinojosa CA, *et al*. A non-human primate model of severe pneumococcal pneumonia. *PLoS One* 2016; **11:** 0166092**.**
63. Anderson R, Feldman C. Pneumolysin as a potential therapeutic target in severe pneumococcal disease. *J Infect* 2017; **74:** 527 – 44.

64. Anderson R, Feldman C. Review manuscript: Mechanisms of platelet activation by the pneumococcus and the role of platelets in community-acquired pneumonia. *J Infect* 2017; **75:** 473 – 85.
65. Anderson R, Nel JG, Feldman C. Multifaceted role of pneumolysin in the pathogenesis of myocardial injury in community-acquired pneumonia. *Int J Mol Sci* 2018; **19**:1147.
66. Iovino F, Hammarlöf DL, Garriss G, Brovall S, Nannapaneni P, Henriques-Normark B. Pneumococcal meningitis is promoted by single cocci expressing pilus adhesin RrgA. *J Clin Invest* 2016; **126:** 2821 – 6.
67. Iovino F, Engelen-Lee JY, Brouwer M, *et al.* pIgR and PECAM-1 bind to pneumococcal adhesins RrgA and PspC mediating bacterial brain invasion. *J Exp Med* 2017; **14:** 1619 – 30.
68. Brissac T, Shenoy AT, Patterson LA, Orihuela CJ. Cell invasion and pyruvate oxidase-derived H_2O_2 are critical for *Streptococcus pneumoniae*-mediated cardiomyocyte killing. *Infect Immun* 2018; **86:** e00569-17.
69. Shenoy AT, Brissac T, Gilley RP, *et al. Streptococcus pneumoniae* in the heart subvert the host response through biofilm-mediated resident macrophage killing. *PLoS Pathog* 2017; **13:** e1006582.
70. Alhamdi Y, Neill DR, Abrams ST, *et al.* Circulating pneumolysin is a potent inducer of cardiac injury during pneumococcal infection. *PLoS Pathog* 2015; **11:** e1004836.
71. Shenoy AT, Beno SM, Brissac T, Bell JW, Novak L, Orihuela CJ. Severity and properties of cardiac damage caused by *Streptococcus pneumoniae* are strain dependent. *PLoS One* 2018; **13:** e0204032.

72. Surve MV, Bhutda S, Datey A, *et al.* Heterogeneity in pneumolysin expression governs the fate of *Streptococcus pneumoniae* during blood-brain barrier trafficking. *PLoS Pathog* 2018; **14:** e1007168.
73. Medina E, Rohde M, Chhatwal GS. Intracellular survival of *Streptococcus pyogenes* in polymorphonuclear cells results in increased bacterial virulence. *Infect Immun* 2003; **71:** 5376–80.
74. Osterlund A, Popa R, Nikkilä T, Scheynius A, Engstrand L. Intracellular reservoir of *Streptococcus pyogenes in vivo*: a possible explanation for recurrent pharyngotonsillitis. *Laryngoscope* 1997; **107:** 640–7.
75. Bastiat-Sempe B, Love JF, Lomayesva N, Wessels MR. Streptolysin O and NAD-glycohydrolase prevent phagolysosome acidification and promote group A *Streptococcus* survival in macrophages. *MBio* 2014; **5:** e1690-14.
76. O'Neill AM, Thurston TL, Holden DW. Cytosolic replication of group A *Streptococcus* in human macrophages. *MBio* 2016; **7:** e00020-16.
77. Fischetti VA, Dale JB. One more disguise in the stealth behavior of *Streptococcus pyogenes. MBio* 2016; **7:** e00661-16.
78. Ercoli G, Fernandes VE, Chung WY, *et al.* Intracellular replication of *Streptococcus pneumoniae* inside splenic macrophages serves as a reservoir for septicaemia. *Nat Microbiol* 2018; **3:** 600–10.
79. Subramanian K, Neill DR, Malak H, Spelmink L, Khandaker S, Dalla Libera Marchiori G, Dearing E, Kirby A, Yang M, Achour A, Nilvebrandt J, Nygren P-Å, Plant L, Kadioglu A, Henriques-Normark B. Pneumolysin binds to the mannose-receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival. *Nat Microbiol* 2018 [in Press].

80. Kenny GE, Wentworth BB, Beasley RP, Foy HM. Correlation of circulating capsular polysaccharide with bacteremia in pneumococcal pneumonia. *Infect Immun* 1972; **6:** 431–7.
81. Coonrod JD, Drennan DP. Pneumococcal pneumonia: capsular polysaccharide antigenemia and antibody responses. *Ann Intern Med* 1976; **84:** 254–60.
82. Schaffner A, Michel-Harder C, Yeginsoy S. Detection of capsular polysaccharide in serum for the diagnosis of pneumococcal pneumonia: clinical and experimental evaluation. *J Infect Dis* 1991; **163:** 1094–102.
83. Boersma WG, Löwenberg A, Holloway Y, Kuttschrütter H, Snijder JA, Koëter GH. Pneumococcal antigen persistence in sputum from patients with community-acquired pneumonia. *Chest* 1992; **102:** 422–7.
84. Van der Auwera P, André A, Bulliard G, *et al.* Comparison of latex agglutination and counterimmuno-electrophoresis in the diagnosis of acute *Streptococcus pneumoniae* infections. *Eur J Clin Microbiol* 1983; **2:** 534–40.
85. Marcos MA, Jiménez de Anta MT, de la Bellacasa JP, *et al.* Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. *Eur Respir J* 2003; **21:** 209–14.
86. Andreo F, Prat C, Ruiz-Manzano J, *et al.* Persistence of *Streptococcus pneumoniae* urinary antigen excretion after pneumococcal pneumonia. *Eur J Clin Microbiol Infect Dis* 2009; **28:** 197–201.
87. Oliver MB, van der Linden MPG, Küntzel SA, Saad JS, Nahm MH. Discovery of *Streptococcus pneumoniae* serotype 6 variants with glycosyltransferase synthesizing two differing repeating units. *J Biol Chem* 2013; **288:** 25976–85.

88. Olaya-Abril A, Prados-Rosales R, McConnell MJ, *et al.* Characterization of protective extracellular membrane-derived vesicles produced by *Streptococcus pneumoniae. J Proteomics* 2014; **106:** 46–60.
89. Codemo M, Muschiol S, Iovino F, *et al.* Immunomodulatory effects of pneumococcal extracellular vesicles on cellular and humoral host defences. *MBio* 2018; **9:** e00559-18.
90. Jhelum H, Sori H, Sehgal D. A novel extracellular vesicle-associated endodeoxyribonuclease helps *Streptococcus pneumoniae* evade neutrophil extracellular traps and is required for full virulence. *Sci Rep* 2018; **8:** 7985.
91. Nerlich A, Mieth M, Letsiou E, *et al.* Pneumolysin induced mitochondrial dysfunction leads to release of mitochondrial DNA. *Sci Rep* 2018; **8:** 182.
92. Naucler P, Darenberg J, Morfeldt E, Ortqvist A, Henriques Normark B. Contribution of host, bacterial factors and antibiotic treatment to mortality in adult patients with bacteraemic pneumococcal pneumonia. *Thorax* 2013; **68:** 571 – 9.
93. Restrepo MI, Faverio P, Anzueto A. Long-term prognosis in community-acquired pneumonia. *Curr Opin Infect Dis* 2013; **26:** 151 – 8.
94. Yende S, Angus DC, Ali IS, *et al.* Influence of comorbid conditions on long-term mortality after pneumonia in older people. *J Am Geriatr Soc* 2007; **55:** 518–25.
95. Adamuz J, Viasus D, Jiménez-Martínez E, *et al.* Incidence, timing and risk factors associated with 1-year mortality after hospitalization for community-acquired pneumonia. *J Infect* 2014; **68:** 534–41.
96. van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, Frölich M. Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet* 1998; **351:** 950–3.

97. Kellum JA, Kong L, Fink MP, *et al.* Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med* 2007; **167:** 1655–63.
98. Yende S, D'Angelo G, Kellum JA, *et al.* Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *Am J Respir Crit Care Med* 2008; **177:** 1242–7.
99. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol* 2018; **14:** 121–37.
100. Paats MS, Bergen IM, Hanselaar WEJJ, *et al*. Local and systemic cytokine profiles in non-severe and severe community-acquired pneumonia. *Eur Respir J* 2013; **41:** 1378 – 85.
101. Yende S, D'Angelo G, Mayr F, *et al* for the GenIMS Investigators. Elevated hemostasis markers after pneumonia increases one-year risk of all-cause and cardiovascular deaths. *PLoS One* 2011; **6:** e22847.
102. Kale S, Yende S, Kong L, *et al* for the GenIMS Investigators. The effects of age on inflammatory and coagulation-fibrinolysis response in patients hospitalized for pneumonia. *PLoS One* 2010; **5:** e13852.
103. Reade MC, Yende S, D'Angelo G, *et al* for the GenIMS Investigators. Differences in immune response may explain lower survival among older men with pneumonia. *Crit Care Med* 2009; **37:** 1655-62.
104. Rajas O, Ortega-Cómez M, Román MG, *et al*. The incidence of cardiovascular events after hospitalization due to CAP and their association with different inflammatory markers. *BMC Pulm Med* 2014; **14:** 197.

105. Zemmour H, Planer D, Magenheim J, *et al.* Non-invasive detection of human cardiomyocyte death using methylation patterns of circulating DNA. *Nat Commun* 2018; **9:** 1443.

106. Tejera A, Santolaria F, Diez ML, *et al.* Prognosis of community acquired pneumonia (CAP): value of triggering receptor expressed on myeloid cells-1 (TREM-1) and other mediators of the inflammatory response. *Cytokine* 2007; **38:** 117 – 23.

107. Kouassi KT, Gunasekar P, Agrawal DK, Jadhav GP. TREM-1; is it a pivotal target for cardiovascular diseases? *J Cardiovasc Dev Dis* 2018; **5:** E45.

108. Peters RP, de Boer RF, Schuurman T, *et al. Streptococcus pneumoniae* DNA load in blood as a marker of infection in patients with community-acquired pneumonia. *J Clin Microbiol* 2009; **47:** 3308–12.

109. Lamontagne F, Garant MP, Carvalho JC, Lanthier L, Smieja M, Pilon D. Pneumococcal vaccination and risk of myocardial infarction. *CMAJ* 2008; **179**: 773 – 7.

110. Hung IFN, Leung AYM, Chu DWS, *et al*. Prevention of acute myocardial infarction and stroke among elderly persons by dual pneumococcal and influenza vaccination: a prospective cohort study. *Clin Infect Dis* 2010; **51**: 1007 – 16.

111. Tseng HF, Slezak JM, Quinn VP, Sy LS, Van den Eeden SK, Jacobsen SJ. Pneumococcal vaccination and risk of acute myocardial infarction and stroke in men. *JAMA* 2010; **303**: 1699 – 706.

112. Vila-Corcoles A, Ochoa-Gondar O, Rodriguez-Blanco T, *et al*. Clinical effectiveness of pneumococcal vaccination against acute myocardial infarction and stroke in people over 60 years: the CAPAMIS study, one-year follow-up. *BMC Public Health* 2012; **12**: 222.

113. Eurich DT, Johnstone JJ, Minhas-Sandhu JK, Marrie TJ, Majumdar SR. Pneumococcal vaccination and risk of acute coronary syndromes in patients with pneumonia: population-based cohort study. *Heart* 2012; **98**: 1072 – 7.

114. Ciszewski A. Cardioprotective effect of influenza and pneumococcal vaccination in patients with cardiovascular diseases. *Vaccine* 2018; **36:** 202 – 6.

115. Ren S, Newby D, Li SC, *et al*. Effect of the adult pneumococcal polysaccharide vaccine on cardiovascular disease: a systematic review and meta-analysis. *Open Heart* 2015; **2**: e000247.

116. Vlachopoulos CV, Terentes-Printzios DG, Aznaouridis KA, Pietri PG, Stefanadis CI. Association between pneumococcal vaccination and cardiovascular outcomes: a systematic review and meta-analysis of cohort studies. *Eur J Prev Cardiol* 2015; **22**: 1185 – 99.

117. Ren S, Hure A, Peel R, *et al*. Rationale and design of a randomized controlled trial of pneumococcal polysaccharide vaccine for prevention of cardiovascular events: The Australian study for the prevention through immunization of cardiovascular events (AUSPICE). *Am Heart J* 2016**; 177**: 58 – 65.

118. Bonten MJ, Huijts SM, Bolkenbaas M, *et al.* Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 2015; **372:** 1114 – 25.

119. Galanis I, Lindstrand A, Darenberg J, *et al.* Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm, Sweden. *Eur Respir J* 2016; **47:** 1208 – 18.

120. Naucler P, Galanis I, Morfeldt E, Darenberg J, Örtqvist Å, Henriques-Normark B. Comparison of the impact of pneumococcal conjugate vaccine 10 or pneumococcal conjugate vaccine 13 on invasive pneumococcal disease in equivalent populations. *Clin Infect Dis* 2017; **65:** 1780 – 9.

121. Martens P, Worm SW, Lundgren B, Konradsen HB, Benfield T. Serotype-specific mortality from invasive *Streptococcus pneumoniae* disease revisited. *BMC Infect Dis* 2004; **4:** 21.

122. Brueggemann AB, Peto TE, Crook DW, Butler JC, Kristinsson KG, Spratt BG. Temporal and geographic stability of the serogroup-specific invasive disease potential of *Streptococcus pneumoniae* in children. *J Infect Dis* 2004; **190:** 1203 – 11.

123. Weinberger DM, Harboe ZB, Sanders EA, *et al.* Association of serotype with risk of death due to pneumococcal pneumonia: a meta-analysis. *Clin Infect Dis* 2010; **51:** 692 – 9.

124. Browall S, Backhaus E, Naucler P, *et al.* Clinical manifestations of invasive pneumococcal disease by vaccine and non-vaccine types. *Eur Respir J* 2014; **44:** 1646 – 57.

125. Kobayashi M, Bennett NM, Gierke R *et al.* Intervals between PCV13 and PPSV23 vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2015; **64:** 944 – 7.

126. Feldman C, Anderson R. Review: current and new generation pneumococcal vaccines. *J Infect* 2014; **69:** 309 – 25.

127. Daniels CC, Rogers PD, Shelton CM. A review of pneumococcal vaccines: current polysaccharide vaccine recommendations and future protein antigens. *J Pediatr Pharmacol Ther* 2016; **21:** 27 – 35.

128. Naghavi M, Barlas Z, Siadaty S, Naguib S, Madjid M, Casscells W. Association of influenza vaccination and reduced risk of recurrent myocardial infarction. *Circulatio*n 2000; **102**: 3039 – 45.

129. Ciszewski A, Bilinska ZT, Brydak LB, *et al*. Influenza vaccination in secondary prevention from coronary ischaemic events in coronary artery disease: FLUCAD study. *Eur Heart J* 2008; **29**: 1350 – 8.

130. Gwini SM, Coupland CAC, Siriwardena AN. The effect of influenza vaccination on risk of acute myocardial infarction: self-controlled case-series study. *Vaccine* 2011; **29**: 1145 – 9.

131. Macintyre CR, Heywood AE, Kovoor P, *et al*. Ischaemic heart disease, influenza and influenza vaccination: a prospective case control study. *Heart* 2013; **99**: 1843 – 8.

132. Chiang MH, Wu HH, Shih CJ, Chen YT, Kuo SC, Chen TL. Association between influenza vaccination and reduced risks of major adverse cardiovascular events in elderly patients. *Am Heart J* 2017; **193**: 1 – 7.

133. Johnstone J, Loeb M , Teo KK, *et al*. Influenza vaccination and major adverse vascular events in high-risk patients. *Circulation* 2012; **126**: 278 – 86.

134. Heffelfinger JD, Heckbert SR, Psaty BM, *et al*. Influenza vaccination and risk of incident myocardial infarction. *Hum Vac*cin 2006; **2**: 161 – 6.

135. Jackson LA, Yo O, Heckbert SR, *et al*. Influenza vaccination is not associated with a reduction in risk of recurrent coronary events. *Am J Epidemiol* 2002; **156**: 634 – 40.

136. Lavallée PC, Labreuche J, Fox KM, *et al*. Influenza vaccination and cardiovascular risk in patients with recent TIA and stroke. *Neurology* 2014; **82**: 1905-13.

137. MacIntyre CR, Mahimbo A, Moa AM, Barnes M. Influenza vaccine as a coronary intervention for prevention of myocardial infarction. *Heart* 2016; **102**: 1953 – 6.

138. Hebsur S, Vakil E, Oetgen WJ, Kumar PN, Lazarous DF. Influenza and coronary artery disease: exploring a clinical association with myocardial infarction. *Rev Cardiovasc Med* 2014; **15**: 168 – 75.

139. Clar C, Oseni Z, Flowers N, Keshtkar-Jahromi M, Rees K. Influenza vaccines for preventing cardiovascular disease. *Cochrane Database Syst Rev* 2015, **5**: CD005050.

140. Fröbert O, Götberg M, Angerås O*, et al*. Design and rationale for the influenza vaccination After Myocardial Infarction (IAMI) trial. A registry-based randomized clinical trial. *Am Heart J* 2017; **189**: 94 – 102.

141. Christenson B, Hedlund J, Lundbergh P, Örtqvist Å. Additive preventive effect of influenza and pneumococcal vaccines in elderly persons. *Eur Respir J* 2004; **23**: 363 – 8.

142. Gilchrist SAN, Nanni A, Levine O. Benefits and effectiveness of administering pneumococcal polysaccharide vaccine with seasonal influenza vaccine: An approach for policymakers. *Am J Public Health* 2012; **102**: 596 – 605.

143. Zhang YY, Tang XF, Du CH, Wang BB, Bi ZW, Dong BR.Comparison of dual influenza and pneumococcal polysaccharide vaccination with influenza vaccination alone for preventing pneumonia and reducing mortality among the elderly: A meta-analysis. *Hum Vaccin Immunother* 2016; **12**: 3056 – 64.

144. Smeeth L, Thomas SL, Hall SJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med* 2004; **351**: 2611 – 8.