Review Article

Current and emerging evidence for immunomodulatory therapy in community-acquired pneumonia

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Abstract: Community-acquired pneumonia (CAP) is the most common infectious disease related cause of death worldwide despite the use of effective antimicrobials. Much of the morbidity and mortality seen in CAP patients at high risk of death has been attributed to exaggerated host responses that result in bystander tissue damage and organ failure. Therefore there is great need to further understand the effect of hyperinflammatory phenotypes on CAP outcomes and develop adjuvant therapy that can attenuate excessive inflammatory responses without compromising host defense. Furthermore, there is growing concern regarding the development of antimicrobial resistance and recent research aims to modulate immune mechanisms that boost pathogen killing and clearance. In this review we summarize the growing body of evidence for the use of adjuvant immunomodulators in the treatment of CAP and highlight emerging immunomodulators that have been tested in pre-clinical studies, which need to be evaluated and developed for clinical trials. In summary, current evidence supports the use of macrolide combination antibiotic therapy and unless contraindicated continuation of pre-admission statin and antiplatelet therapy. Corticosteroids are beneficial in the context of septic shock and critical illness related adrenal insufficiency and may be of benefit to individuals with severe CAP and a hyperinflammatory phenotype given the potential for improving patient-centered and economic outcomes with negligible adverse effects. Despite much promise in pre-clinical work, many clinical trials of drugs targeting the coagulation pathways have unfortunately failed to demonstrate clinical benefits in humans. Results of trials evaluating aspirin, intravenous immunoglobulin (IVIg) and thrombomodulin are awaited and may yet influence practice, whilst further identification of inflammatory phenotypes will in the future allow personalized approaches and identify subgroups of patients that may respond to adjuvants that have previously not demonstrated favorable outcomes when used in heterogeneous cohorts.

Keywords: Community-acquired pneumonia (CAP); immunomodulation; inflammation; coagulation; corticosteroids; macrolides; statins; anti-platelets; proteinase-activated receptors; pattern recognition receptors; intravenous immunoglobulins (IVIg)

Introduction

Despite effective antimicrobials, community-acquired pneumonia (CAP) remains a significant cause of morbidity and mortality (1). In 2012 the annual European incidence of CAP was 150–170 episodes per 100,000 people (2). The incidence progressively rises in older age groups, to roughly 2% in those aged 81 years and over compared to 0.2% in those aged 31–40 years, and in more deprived social groups with pneumonia around 45% more common in the most deprived social quintile compared to the least (3). Between 22% and 42% of those with CAP require hospital admission (4), accounting in 2015–2016 for 248,916 out of 16.3 million UK National Health Service (NHS) hospital admissions (5). This compares with 674,000 hospital admissions due to pneumonia of a total of 12.2 million in the US (6). Pneumonia and influenza were the 6th and 4th commonest causes of death in 2013 in UK males and females respectively, accounting for 5–6% of deaths annually or an age-standardized mortality rate per million population of 614 in males and 473 in females (7). In the US the adjusted figure is 151 per million population overall, with a similar male predominance, causing 2.1% of all deaths, ranking 8th commonest (8).

Austrian and Gold demonstrated the significant improvement in outcomes following the introduction of antimicrobial therapy for uncomplicated bacteremic pneumococcal pneumonia, with overall mortality reduced from 80% to 17% (9). However despite advances over the subsequent 50 years, mortality remains high at 12% for bacteremic pneumococcal pneumonia (10), and as high as 21–58% amongst the severe CAP subgroup (11). Therefore, there is great need to focus our research efforts on identifying novel therapeutic strategies that can be used in combination with antimicrobials to reduce mortality from CAP.

A role for immunomodulation

With current culture-based microbiological techniques often the causative organism is not identified, but when detected the commonest causative community-acquired pathogen is *Streptococcus pneumoniae*, and viruses including Influenza are increasingly being recognized as a cause of primary viral pneumonia or secondary bacterial pneumonia (1,12). Legionella species, *Staphylococcus aureus*, enteric bacteria and other organisms, for example *Klebsiella pneumoniae*, account for a disproportionate number of

ICU admissions compared to their overall incidence. Antimicrobial resistance varies markedly, with rates of resistance for *S. pneumoniae* over 30% in Spain and Greece but below 3% in Germany (13). However studies suggest that clinically-significant antibiotic resistance is a rare cause of treatment failure in CAP and is not an independent risk factor for poor prognosis (14). In contrast, it has been shown that patients with a delayed time to clinical stability over 3 days have a persistently elevated level of plasma cytokines, compared to the rapid decline seen in those who improve quickly (15). Thus, these factors combined drive interest in the impact of underlying host factors in the response to infection and the potential to modulate these to improve outcomes. Much as in the new sepsis guidelines (16), where the stress is on the dysregulated host response to infection defining sepsis and driving the associated mortality, a similar theory is appropriate to severe CAP where excess local inflammation causing tissue destruction and alveolar-endothelial capillary barrier disruption precipitates lung injury (17), systemic inflammation underpins sepsis (18), and activation of coagulation triggers disseminated intravascular coagulation (DIC) (19), with the latter two causing microthrombi and microvascular dysfunction leading to multiorgan failure. Likewise, the challenges in advancing management and improving survival come firstly in identifying this cohort early and differentiating from those with CAP with a high likelihood of survival from the outset, and secondly in identifying the pathophysiological mechanisms defining this cohort and appropriately targeting these.

Immune, inflammatory and coagulation response to respiratory tract infection

The immune response to respiratory pathogens is complex and is reviewed in detail elsewhere (20-23). Here we provide a concise summary to highlight important immune and inflammatory pathways particularly those related to innate immunity. Alveolar macrophages are resident in the lung and play important roles in homeostasis, prevention of inflammation through an inhibitory interaction with the epithelium, and in daily clearance of small numbers of pathogens that are inhaled or aspirated. However, in the presence of large numbers of pathogens they become overwhelmed and are unable to control the infection, requiring the recruitment of immune cells (24). During exposure to respiratory tract pathogens, pathogen-derived pathogen-associated molecular patterns (PAMPS) and host-

derived damage-associated molecular patterns (DAMPS) bind Toll-like receptors (TLRs) on epithelial cells, and alveolar macrophages, causing release of nuclear factor kappa B (NF-κB)-transduced pro-inflammatory cytokines, including tumour necrosis factor (TNF), interleukin (IL)-1β, and IL-6 (25), and chemokines such as CXCL8 and CCL2 to recruit neutrophils and monocytes, respectively, from the pulmonary circulation to the airspaces. Neutrophils kill the pathogens primarily via phagocytosis, whereby internalization of the pathogen into a phagosome results in degranulation, release of antibacterial and lytic enzymes and production of reactive oxygen species in the oxidative burst response (15). Additionally, neutrophils can release DNA-based neutrophil extracellular traps, which can trap pathogens, kill bacteria via histones and antimicrobial granular proteinases and opsonize fungi (26). Once activated, neutrophil apoptosis and subsequent clearance by macrophages is necessary for de-escalation of the response. Dendritic cells exposed to pathogens initiate the adaptive immune response, migrating to the lymph nodes to recruit helper T lymphocytes and induce memory T lymphocytes.

Although this inflammatory response is essential for control of the infection and clearance of pathogens, an uncontrolled or exaggerated inflammatory response can result in bystander tissue injury. Therefore, an appropriate balance between cytokine production and neutrophil activation successfully combating bacterial infection and overproduction and dysregulation resulting in excessive lung injury is key. When measured, persistent cytokine elevation, particularly IL-6 but also IL-10, correlates with mortality even following hospital discharge (27,28). Further, it has been demonstrated that as compared to patients with non-severe CAP, patients with severe CAP at the time of hospital admission have significantly elevated plasma levels contributing to an exaggerated systemic inflammatory response (29). This is in keeping with studies suggesting that failure of neutrophil depriming in the lungs results in higher systemic levels of activated neutrophils in patients with the acute respiratory distress syndrome (ARDS) (18).

Importantly, there is a close relationship between inflammation and coagulation allowing haemostatic containment to contribute to the initial host response to infection. Activation of the coagulation cascade by tissue injury or inflammation secondary to infection occurs by stimulating the expression of tissue factor (TF) on the surface of mononuclear cells, fibroblasts, alveolar and epithelial cells (30). When TF expressed on cells is exposed to blood it activates and binds to the inactive zymogen factor VII (FVII). The TF-FVIIa complex then initiates the TF dependent pathway of coagulation by activating FX and binding to FXa, forming the TF-FVIIa-FXa ternary complex, which together with thrombin-induced positive feedback activation of FV and FVIII, results in significant thrombin generation and fibrin cross-linking. Platelets, activated via thrombin or directly via platelet-activating factor, are also involved in both coagulation and inflammation, expressing P-selectin which both binds neutrophils and augments macrophage TF expression via NF-κB (31,32).

Linking inflammation and coagulation are the protease-activated receptors (PARs), a group of seven transmembrane G protein-coupled receptors which undergo proteolytic cleavage of the extracellular N-terminus and the unmasking of a previously cryptic tethered ligand, which interacts with the second extracellular loop of the receptor resulting in conformational change of the receptor and initiates cell signaling via the recruitment of heterotrimeric G proteins (30). Thrombin acts via PAR-1 to induce the release of pro-inflammatory cytokines (such as IL-1β) and chemokines (including CXCL1, CCL2 and CCL7) (33), and at high concentrations stimulates platelets via PAR-1 and PAR-4 to release further platelet agonists, chemokines and growth factors, potentiating both inflammation and coagulation and contributing to endothelial disruption (34). In contrast, epithelium-bound activated protein C (APC) acts via PAR-1 to downregulate inflammation and improve host defense to endotoxins (35,36).

Coagulation is normally regulated by antithrombin, activation of protein C, and TF pathway inhibitor (TFPI). However, during severe infection these mechanisms are impaired. Consumption and diminished production reduce the levels of the former two, activated neutrophils produce elastases degrading antithrombin and TFPI, downregulation of endothelial thrombomodulin prevents activation of protein C, and increased plasma C4b binding protein levels as an acute phase reactant result in a relative protein S deficiency. Furthermore, there is an increase in plasminogen activator inhibitor type-1 (PAI-1) resulting in reduced plasminogen activation and hence reduced fibrinolysis (37,38).

Targeting inflammation pathways

Macrolides

In addition to antimicrobial effects on both typical (e.g.,

S. pneumoniae) and atypical (e.g., *Mycoplasma pneumoniae*) respiratory tract pathogens, macrolides are known to impact on the host-pathogen interaction both directly and by immunomodulatory effects via multiple mechanisms including altering the balance in favour of anti-inflammatory cytokines, facilitating phagocytosis of apoptotic cells by alveolar macrophages, reducing recruitment and adhesion of both neutrophils and T-cells, and increasing neutrophil degranulation thereby enhancing bactericidal activity (39). First noted following striking improvement in outcomes of patients with diffuse panbronchiolitis (40), macrolides are now frequently used for long-term reduction of inflammation in bronchiectasis and chronic obstructive pulmonary disease as well as acutely for their immunomodulatory properties in CAP.

In a recent meta-analysis of 10,000 critically ill patients with CAP caused by a range of pathogens, macrolide therapy reduced the overall relative risk of mortality by 18% versus non-macrolide containing antibiotic regimens (41). A trend towards reduced mortality was maintained in subgroup analyses of patients requiring mechanical ventilation and in those managed with macrolide/beta-lactam rather than fluoroquinolone/beta-lactam combination therapy, though not when the causative pathogen was limited to *S. pneumoniae*, when only prospective studies were included, or in a smaller group with septic shock. This effect persists regardless of the presence of *ex vivo* macrolide resistance (42). Furthermore, observational data from the US, Europe and Latin America shows a significant reduction in mortality in ICU patients managed with a macrolide with an odds ratio of 0.45 (95% CI, 0.31–0.66) (43). This effect was lost when assessing patients admitted to ward level care. Similarly, a recent prospective study assessing patients admitted with CAP but not requiring ICU showed beta-lactam monotherapy to be non-inferior to either beta-lactam/macrolide combination or fluoroquinolone monotherapy (44), suggesting that macrolides are beneficial in those with severe inflammatory disease and increased risk of death.

Corticosteroids

Corticosteroids may be helpful in managing bronchospasm related to underlying airways disease or de novo due to the infection itself, or may be beneficial in patients in whom there is an associated critical illness-related corticosteroid insufficiency (CIRCI) (45,46). Moreover, the wider role of steroids as an adjunct in abrogating the inflammatory

response associated with poorer outcomes in CAP is of significant interest. A number of meta-analyses have evaluated the available evidence. A Cochrane review identified six studies up to 2010 totaling 437 participants (47). These are disparate trials studying variously adults and children, differing corticosteroid regimens including with inhaled budesonide, and a range of outcomes. The overall conclusion was that despite failing to improve mortality, steroids may accelerate time to clinical stability, improve oxygenation, reduce the need for mechanical ventilation and decrease the rate of relapse. However the data quality is poor with only two studies deemed to be of high quality and therefore it is difficult to extrapolate findings to clinical practice. More promisingly, analysis of 9 randomized controlled trials (RCTs) of 1,001 patients with either severe or mixed CAP managed with varying corticosteroid regimens up to 2011 showed no overall mortality benefit, but in subgroup analyses a reduction in mortality was seen in patients with severe CAP (odds ratio 0.26) and in those given steroids for longer than 5 days (odds ratio 0.51) (48).

Much of the criticism of the available data revolves around either the wrong patient cohort being studied, who were unlikely to reflect a hyperinflammatory phenotype, or an inappropriately large dose or short duration corticosteroid regimen being used. One of the earlier RCTs demonstrating a benefit addressed this by enrolling Italian patients with severe CAP as defined by the old American Thoracic Society (ATS) criteria and an average C-reactive protein (CRP) of 290 mg/L (placebo) or 550mg/L (intervention), managed with hydrocortisone as a 200 mg bolus followed by 240 mg/d infusion for 7 days (49). The trial was actually stopped at the interim analysis because of favorable outcomes in the intervention arm, with hydrocortisone after 8 days effecting a significant improvement in ratio of arterial oxygen partial pressure to fraction of inspired oxygen (paO2:FiO2), and a significant reduction in radiographic infiltrates, CRP, multiorgan dysfunction scores, and incidence of delayed septic shock. However only 46 patients were studied, there was an albeit non-significant increase in comorbidities in the placebo arm and cytokine levels were not measured.

A more recent RCT randomized 120 Spanish patients with severe CAP as defined by the modified Infectious Diseases Society of America (IDSA)/ATS criteria or pneumonia severity index (PSI) class V and CRP greater than 150 mg/L to receive methylprednisolone 0.5 mg/kg 12 hourly for 5 days or placebo (50). Whilst the primary outcome of a reduction in treatment failure was achieved,

this was the result of a significant reduction in late (72–120 h) radiographic progression in the intervention arm, which the authors argue is a surrogate for mortality requiring fewer participants to achieve the necessary power. There were no statistically significant differences in other measures of early or late treatment failure or in secondary outcomes including time to clinical stability, ICU and hospital length of stay, and in-hospital mortality. Furthermore, except for a significant reduction in CRP at day 3 in the intervention arm, there was no significant difference between arms at either day 3 or 7 of procalcitonin, IL-6, IL-8 or IL-10. Limitations of the study include the non-significantly higher rate of chronic pulmonary disease and viral aetiologies in the intervention arm, the low rates of macrolide usage and delays to administering first dose of antibiotics in both groups.

In contrast, a contemporary study included 785 Swiss patients admitted with CAP of all severities with similar average baseline CRP of around 160 mg/L, randomising them to receive either prednisone 50 mg daily for 7 days or placebo (51). Prednisone reduced time to clinical stability from 4.4 to 3.0 days with no increase in pneumonia-related complications to 30 days. It also resulted in a significant reduction in CRP concentrations at days 3, 5 and 7, but this did not impact on other outcomes including mortality, length of ICU and hospital stay, and readmission.

The most recent meta-analysis includes these studies amongst 9 RCTs and 6 cohort studies, finding that the cumulative data available still lacks power to make significant conclusions (52). There was no difference in mortality amongst the RCTs, nor amongst the RCTs and cohort studies enrolling patients with severe CAP only. Pooling of data from 3 RCTs in which patients were given a loading dose of steroids did show a mortality benefit with a relative risk of 0.23 (95% CI, 0.09–0.63), but the overall dataset is small. Despite the inconsistent reporting of data, corticosteroids did tend to reduce length of stay, duration of intravenous antibiotics and time to clinical stability, and there was a signal of reduced rates of ARDS. Finally the largest dataset is an observational study of 6,925 patients with pneumonia, in whom a mortality benefit appeared to be restricted to cases complicated by septic shock (53), and reflects previous findings for the use of corticosteroids in sepsis (54).

Despite the lack of efficacy in reducing mortality, there is a signal that corticosteroids may reduce other clinically relevant measures including lengths of stay and time to clinical stability, which are beneficial both to the patient

and in terms of health economics. It is reassuring that aside from increased hyperglycemia in some studies, there are no significant adverse effects associated with steroid use either in terms of pneumonia-related complications or systemic sequelae. However, until a larger body of good quality evidence is available many clinicians will still opt not to use them in the absence of septic shock. Further research into corticosteroid use is still required particularly as it remains unclear whether to target the severe CAP subgroup as currently defined or all patients with CAP, what the optimum corticosteroid and dosing regimen is, and if more specific patient phenotyping is required to identify corticosteroid responders.

Statins

Beyond lipid-lowering properties, statins have immunomodulatory effects including improvement of endothelial dysfunction, downregulation of endothelial adhesion molecules, reduced cytokine production and reduced neutrophil recruitment (55,56). This is supported *in vivo* by data showing a reduction in IL-6 and TNF in hospitalized patients with bacterial infections managed with simvastatin compared to placebo in addition to standard care (57). There is however a conflicting body of evidence for their impact in CAP with population-based studies in primary care suggesting a lower risk of developing pneumonia in patients taking statins (58) and similar observational studies suggesting a reduced 30-day mortality in patients admitted with pneumonia (59). However, a more recent US primary care case-control study of over 65s found no reduction in risk of CAP associated with statins (60). The study excluded nursing home residents, whom the authors suggest are both at a higher risk of developing pneumonia but also perhaps less likely to be prescribed a statin, and therefore previous studies not doing likewise may have been subject to 'healthy user' bias.

Despite adjusting for multiple confounders, there is an argument that the benefit seen is simply in the reduction of secondary cardiovascular events due to supply and demand mismatch in myocardial perfusion (61). However in a study of patients hospitalized with an acute coronary syndrome or ischemic stroke, commencing a statin within 90 days of discharge was associated with a significant reduction in the risk of sepsis, severe sepsis and fatal sepsis with hazard ratios of 0.81, 0.83 and 0.75 respectively, an effect not seen with other lipid-lowering medications (62).

There is limited data studying statins as an intervention

in CAP and these studies are needed since there is a suggestion from a small non-interventional study that statins may be associated with enhanced efferocytosis (63) which could lead to better recovery from pneumonia. Furthermore, a recently published study demonstrated that high-dose (80 mg) simvastatin enhances the migratory accuracy of neutrophils *in vitro* from elderly individuals with CAP, but only in those with less severe disease (64), corroborating previous findings that statins are not beneficial in severe sepsis and ARDS (65-67). Importantly, it is not known what effect simvastatin-enhanced neutrophil migratory accuracy will have on important clinical outcomes of individuals with CAP and future prospective interventional trials are needed. Monitoring for adverse effect will however be required, since high-dose simvastatin may be associated with detrimental adverse effects such as elevated creatinine kinase or hepatic transaminases as seen in the HARP-2 trial (67).

Intravenous immunoglobulins (IVIg)

Patients with CAP have been found to have lower levels of immunoglobulin (Ig) G and IgA compared to healthy subjects (68). Progressively lower levels of all IgG subsets and IgA correlate with severity of CAP in immunocompetent individuals as determined by need for ICU admission and CURB-65 score, and total IgG is independently associated with need for ICU admission even when accounting for CURB-65 score and cardiorespiratory comorbidities (69). Interestingly there is no difference between patients admitted to the ward and those managed as an outpatient, suggesting that hypogammaglobulinemia may distinguish the ICU cohort (69). Additionally, these deficiencies may persist for as long as 9 months post-infection (70).

The effects of IVIg on outcomes from CAP are not well known and results of the CIGMA study assessing use of IgM-enriched polyclonal IVIg in severe CAP requiring mechanical ventilation are currently awaited (71). In sepsis IVIg may be beneficial by neutralising bacterial toxins, improving bacterial opsonization and modulating complement activation (72). However, current data do not strongly support its use. One meta-analysis found a 21% relative risk reduction for mortality using polyclonal IVIg in patients with sepsis or septic shock (73). However in another study IVIg given on days 0 and 1 in all-cause severe sepsis had no impact on 7- or 28-day mortality, or IL-6 and TNF receptor levels (74).

A contemporary systematic review found that although overall there was a reduction in mortality in trials of polyclonal IVIg, when only high-quality data was included there was no benefit (75). Similarly, a subsequent Cochrane review also showed polyclonal and IgM-enriched IVIg reduced mortality in sepsis and septic shock in adults with a relative risk of 0.81, but that when only 5 trials adjudged to have a low risk of bias were included, no such effect was seen (76).

In Japan, where polyclonal IVIg can be considered for use in sepsis for up to 3 days, a retrospective database analysis of patients with septic shock due to pneumonia requiring mechanical ventilation showed no benefit to IVIg in terms of 28-day or in-hospital mortality, ventilator-free days or catecholamine-free days either in raw data or with propensity matching (77). Immunoglobulin levels are not reported and it is interesting that again use of macrolides was low at around 8% in both groups. This may reflect an overall lack of efficacy or that the wrong cohort was reviewed and that there may be a benefit to IVIg in patients identified by low levels prior to establishment of either respiratory failure requiring mechanical ventilation or sepsis. Such a theory is supported by data suggesting that as with antibiotics in sepsis, efficacy of IVIg is time-dependent (78).

Targeting coagulation pathways

A significant body of evidence from pre-clinical work suggests that targeting coagulation pathways can modulate the inflammatory response to infection. In clinical studies, much of the data evaluates either patients with sepsis rather than CAP per se, or patients developing ARDS. However, given the high proportion of these cohorts with an underlying diagnosis of CAP, these data are useful to extrapolate or conduct retrospective subgroup analyses to inform future research directions in CAP management. Given the concerns regarding adverse bleeding events with systemic administration of anticoagulants, there is a significant body of work looking at nebulized drug delivery to the lungs, which may permit higher therapeutic doses to be delivered to the site of action. The majority of these studies used animal models of *S. pneumoniae* or lipopolysaccharide-induced pneumonia and have demonstrated reduced activation of coagulation and inflammation in the lungs without systemic adverse effects (79). Human studies are therefore greatly desired.

APC [drotrecogin alfa (activated)]

As described above, protein C associated with

thrombomodulin on the epithelial surface is activated by thrombin and regulates coagulation by inhibiting FV and FVIII, promotes fibrinolysis by inhibiting PAI-1, and inhibits macrophage TNFα production. In humans it causes a reduction in d-dimer and IL-6 levels in patients with severe sepsis, implying a downregulation of both coagulation and inflammation pathways (80). The PROWESS trial was terminated early for efficacy, demonstrating a significant reduction in 28-day mortality in patients with severe sepsis managed with adjunctive APC [drotrecogin alfa (activated)] irrespective of baseline levels of protein C, with a relative risk reduction of 19.4% (80). Subgroup analysis showed this benefit was sustained only in those patients with an Acute Physiology and Chronic Health Evaluation (APACHE II) score ≥25 or 2 or more organ failures (81). APC was thus approved for adjunctive use in patients with sepsis and a high risk of death or multiple organ failure and adopted into the surviving sepsis campaign guidelines (82,83). However, the subsequent ADDRESS study showed no overall mortality benefit in patients at lower risk of death and subgroup analysis actually suggested an increased mortality in patients with an APACHE II score ≥25 who were randomized to APC (84). Adverse events were seen in all studies with serious bleeding significantly higher with APC regardless of use of concurrent heparin, in PROWESS at 3.5% compared to 2.0% with placebo, though rates of thrombosis were similar. Furthermore, in the ENHANCE open-label trial, the serious bleeding rate was higher with APC at 5.5% and there was a higher rate of intracranial haemorrhage (85), suggesting that this strategy may lead to harm in some patients. Subsequently therefore, the PROWESS-SHOCK trial was mandated, and found no benefit in 28- or 90-day mortality from APC given to patients with septic shock and signs of hypoperfusion either as a whole or in a priori defined subgroups (86).

A large proportion (54% and 44%) of patients in the PROWESS and PROWESS-SHOCK trials had a focus of infection within the lungs and post hoc analysis of the PROWESS trial assessed the impact of APC in those patients with CAP (87). They were able to identify 35.6% of patients as having sepsis related to CAP, with a slightly higher proportion within the APC arm, of whom around a quarter isolated *S. pneumoniae*. Interestingly, IL-6 levels were higher in patients with *S. pneumoniae* in whom upwards of 60% were bacteremic, roughly double the rate in CAP of other aetiologies, but there was no difference

in either baseline markers of illness severity or mortality. Unadjusted data showed improved 28-day mortality in patients with CAP with a relative risk of 0.72, (95% CI, 0.55–0.94) but there was no statistically significant difference at 90 days. Further subgrouping showed the benefit to be limited at 28 days to CAP patients with APACHE II score ≥25, PSI score ≥4 or requiring both vasopressor and ventilatory support, and by 90 days only those with APACHE II score ≥25 continued to have a mortality benefit. However following PROWESS-SHOCK, drotrecogin alfa (activated) has been withdrawn from the market.

TFPI (tifacogin)

Recombinant TFPI (rTFPI; tifacogin) administered in sepsis may help restore appropriate regulation of coagulation and prevent endothelial injury, which might be particularly beneficial in reducing acute lung injury, although the mechanisms of action are debated. The OPTIMIST phase III trial of rTFPI in severe sepsis showed no overall benefit in patients with an international normalized ratio (INR) ≥1.2 despite evidence of biological activity, although contrary to phase II data there was a benefit in the smaller group of patients with INR <1.2, with increased rates of serious bleeding compared to placebo regardless of INR (88). One explanation for the lack of benefit is that doses causing significant bleeding may be lower than that required to regulate inflammation, thus precluding efficacious doses being administered (89). Post hoc subgroup analyses however highlighted a benefit in patients with documented bacteremia or pneumonia, particularly when concurrent heparin was not administered.

The signal of benefit in pneumonia led to the CAPTIVATE trial assessing 2 doses of rTFPI versus placebo in patients with severe CAP as defined by the IDSA/ATS criteria not on concurrent heparin (90). The higher dose arm was discontinued early due to futility, but in the final results, there was equally no mortality benefit to rTFPI at the OPTIMIST dose regardless of severity as defined by APACHE II or PSI. There was also no difference in risk of deteriorating to require mechanical ventilation, developing ARDS, or developing DIC. It is noteworthy however, that there was a pre-defined protocol for interrupting rTFPI infusion in response to rising INR or falling platelet count and when it is considered that there was no difference in adverse events including

bleeding between the intervention and placebo arms, it may be that this protocol resulted in delivery of inadequate doses thereby protecting patients from harm but reducing efficacy.

Antithrombin III (ATIII)

A number of small studies demonstrated a mortality benefit of ATIII in sepsis, particularly in the more critically unwell and shocked patients (91). The KyberSept phase III trial was therefore undertaken to explore the use of high-dose plasma-derived ATIII in patients with severe sepsis (92). This demonstrated no overall benefit to 28-day mortality, although post hoc subgroup analysis showed an improved 90-day mortality in patients not given concurrent heparin and in those with a higher mortality as predicted by the Simplified Acute Physiology Score (SAPS II). There was also a significantly higher risk of bleeding, exacerbated in the group co-administered heparin. The trial was criticized for failing to enrol patients as critically ill as intended, which given previous data may have had an impact on the expected efficacy, to achieve the intended supraphysiological levels of ATIII, and to protocolize heparin coadministration. A Cochrane review found there to be an associated bleeding risk and the available evidence to be of a low quality and not currently supportive of using ATIII in critically ill patients, including those with sepsis (93). A Japanese retrospective database analysis of ATIII in patients with severe pneumonia and sepsis-associated DIC (note cases not confirmed as community-acquired) has suggested improved 28-day mortality with an adjusted odds ratio of 0.85 (95% CI, 0.75–0.97) in the propensity-matched groups, and additionally increased ventilator-free days (94). However such evidence cannot support widespread use of ATIII in CAP and prospective RCTs are required.

Thrombomodulin

The safety and bioactivity of recombinant thrombomodulin in sepsis associated with DIC has been demonstrated in a phase 2 trial, with a trend of improved mortality particularly in patients with dysfunction of at least one organ system (95). Interestingly case reports and small studies, both retrospective and prospective, suggest a mortality benefit of recombinant thrombomodulin in patients with acute exacerbations of idiopathic interstitial pneumonia (96-98), hinting at a benefit in reducing pulmonary inflammation in addition to effects on DIC. A similar Japanese retrospective

analysis to that for ATIII found no effect on 28-day mortality of recombinant thrombomodulin given to patients with severe CAP and sepsis-associated DIC, compared to a propensity-matched cohort (99) and therefore we now await the results of a phase 3 trial in severe sepsis with coagulopathy which is currently recruiting (100).

Antiplatelet agents

As with statins, it is likely that continuing antiplatelet therapy during CAP helps mitigate the risk of secondary cardiovascular events. Beyond this however, the potential of this drug class to inhibit the contribution of platelets to both excessive coagulation and inflammation in CAP has prompted further investigation. Aspirin downregulates NF-κB, inhibiting the cascade of inflammatory cytokine production (101), acts on endothelial nitric oxide synthase to increase nitric oxide levels impairing neutrophil recruitment and microthrombi formation (102), and induces production of aspirin-triggered lipoxins which exert anti-inflammatory effects (103).

A prospective study of Italian patients with a mean age around 75 years presenting with CAP of all severities stratified by pre-admission use of aspirin found twice as many patients were not taking aspirin, and that patients not taking aspirin were significantly more likely to have severe CAP with evidence of acute lung injury at baseline and more likely to have organ dysfunction, severe sepsis or septic shock (104). Following propensity matching, an increased 30-day mortality was demonstrated in the non-aspirin group with hazard ratio 2.07 (95% CI, 1.08–3.98; P=0.029). A similar prospective analysis of patients with ARDS using multivariate logistic regression analyses demonstrated that patients receiving aspirin either pre-hospitalization or during their admission had a reduced risk of in-ICU mortality with an odds ratio of 0.38 (95% CI, 0.15–0.96; P=0.04) (105). This effect was not noted when patients taking both aspirin and statins were evaluated, suggesting that the benefit is unlikely due to decreased cardiovascular events. Furthermore, two recent meta-analyses of cohort studies agree that antiplatelet agents in critically ill patients are associated with decreased mortality, incidence of ARDS and mechanical ventilation (106,107).

There are a number of trials of aspirin as an intervention currently ongoing, including one assessing the incidence of acute lung injury in medical "at risk" patients given aspirin (108), one assessing oxygenation in patients with established ARDS given aspirin 75 mg (109), and one assessing the efficacy of aspirin 75 mg and 1,200 mg in reducing induced lung inflammation

in healthy volunteers (110). Unfortunately, a RCT designed to assess the mortality benefit of commencing ticagrelor de novo in patients with severe CAP has recently terminated having failed to recruit sufficient patients (111).

Other immunomodulators

Pattern recognition receptors

Pattern recognition receptors including TLRs play a key role in response to infection with PAMP recognition eliciting the initial innate immune response to eradicate a pathogen and PAMP and DAMP recognition contributing to propagation of the inflammatory response. In animal models, different TLRs have been associated with a variety of both infectious and inflammatory or autoimmune diseases. For example, TLR4 has been implicated in endotoxemia-induced systemic inflammation and sepsis, but is required for response to influenza A, *S. pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* infections (112-114). The anti-inflammatory nature of inactivated alveolar macrophages conveys a higher threshold for activation of innate immunity within the respiratory tract. Thus agonism of TLRs may upregulate the response to pathogen exposure helping prevent infection or improve pathogen clearance, whilst antagonism may inhibit the deleterious hyperinflammatory response.

A number of animal models have demonstrated a benefit to such strategies. Separate monoclonal antibodies to TLR2 and TLR4 when administered subcutaneously successfully reduced mortality in polymicrobial models of intra-abdominal sepsis in mice concurrently treated with antibiotics, although the effect was most marked when the antibodies were administered prior to induction of sepsis and was reduced though still statistically significant when given 3 h after (115). However, in humans, in the phase III ACCESS trial, eritoran, a synthetic TLR4 antagonist, failed to show a benefit in reducing mortality in patients with severe sepsis as an adjunct to antibiotics, early goal directed therapy plus/minus APC, regardless of disease severity, site or organism (116). In roughly 50% of cases there was a pulmonary source of infection, but subgroup analysis of this cohort did not demonstrate any benefit. In the phase 1 trial as in the animal model above, when given immediately prior to lipopolysaccharide injection, the TLR4 antagonist successfully attenuated both the clinical symptoms and the elevation in biomarkers of inflammation associated with the

lipopolysaccharide (117). These findings overall suggest that antagonism of TLRs may reduce initiation of the harmful inflammatory cascade, but cannot adequately downregulate this once the process has been triggered, which clearly limits the clinical utility.

Research into TLR agonism has focused on prophylaxis of infection either by enhancing response to vaccination or in lieu of a vaccine. TLR2, TLR4 and TLR5 agonist treatment prior to infection has improved survival in murine models of *S. pneumoniae*, influenza A and *Pseudomonas aeruginosa* pneumonia, respectively (118-121), primarily by promoting neutrophil recruitment thus enhancing pathogen clearance. A similar strategy may possibly have therapeutic implications since the administration of a TLR5 agonist together with antibiotics at 12 h post infection with *S. pneumoniae* enhanced bacterial clearance and reduced lung injury (122). However, murine studies have demonstrated that the magnitude of resistance to infection is less with individual TLR agonism and the effect can be enhanced with synergistic TLR agonist administration (123) or use of aerosolized non-typeable *Haemophilus influenzae* lysate (124). Using this strategy in a murine acute myeloid leukemia model with or without chemotherapy-induced severe neutropenia, a single prophylactic inhaled dose of TLR2/6 and TLR9 agonist was able to clear pulmonary bacterial loads and improve survival in *P. aeruginosa*, *S. pneumoniae* and *Aspergillus fumigatus* infection (125), suggesting that TLR agonism can induce important immune effector functions that are independent of neutrophil recruitment and offers an important potential prophylactic option for immunocompromised hosts at risk of infection. Whilst TLR agonism clearly enhances pathogen clearance in murine models, it is less clear what the impact of TLR agonism on pathogen clearance and lung injury is in clinical practice and the results of human trials are eagerly awaited.

PARs

Given the position of PARs at the interface between coagulation and inflammation, which as discussed is implicated in the injurious host response to an infectious stimulus and the development of ARDS (30,33), these agents may be suitable as adjuvants in the treatment of pneumonia to protect against or reduce lung injury. However, their divergent effects make therapeutic manipulation challenging and further characterization of their effect over time in the context of acute infection is required. In preclinical work, PAR-1 knockout mice had

increased early survival following *S. pneumoniae* pulmonary infection, with reduced pulmonary and blood bacterial loads (126). Furthermore, use of a highly specific PAR-1 antagonist (SCH530348) reduced neutrophil recruitment to the lungs of mice infected with *S. pneumoniae* and decreased alveolar leak without compromising host defense (33). In the context of peritoneal sepsis, further studies using PAR-1 agonists and antagonists have shown a variable mortality effect dependent on the time following infection, with PAR-1 detrimental early on, but beneficial in later stages of infection (127).

More recently the cardiovascular morbidity and mortality associated with pneumonia has been an important focus of research and since PAR-1 antagonism inhibits platelet aggregation in humans, and in the context of *S. pneumoniae* infection PAR-1 antagonism inhibits *in vitro* neutrophil-platelet heterotropic aggregates induced by pneumolysin (34), this method of action could in addition to reducing neutrophilic inflammation offer cardioprotection to infected individuals. An important consideration though is that platelet inhibition increases risk of bleeding. However in the studies that demonstrated that PAR-1 antagonism significantly increased the risk of intracranial haemorrhage in patients with previous stroke, it is important to note that these individuals were already on dual anti-platelet therapy (128). As yet, work in the field of CAP and ARDS remains in the preclinical phase.

Stem cells

Preclinical studies have shown that mesenchymal stem cells (MSCs) have multiple immunomodulatory effects. MSCs are immunoregulatory by direct cell-cell interaction, by paracrine signaling and by generation of regulatory T cells (129), they are immunomodulatory by reprogramming macrophages to an anti-inflammatory IL-10-secreting phenotype (130), and they have both direct and indirect antimicrobial effects (131). They are recruited to sites of active inflammation and their activity can be modulated by inflammatory cytokines, TLRs and bacteria. Additionally, they have only low-level immunogenicity meaning allogeneic MSCs may be a viable therapy without the need for immunosuppression. Animal models appear to demonstrate reduction in bacterial load and attenuation of end-organ damage including to the lung in sepsis models, but with variable effects on mortality, which seem to be influenced by dosing regimen and timing related to the

course of infection (132). In Europe, the SEPCELL project has been established to investigate the potential of stem cell therapy in CAP-induced sepsis in phase Ib/IIa clinical trials and the outcomes of these studies are awaited (133).

Neutralising pneumolysin

Pneumolysin is a pneumococcal virulence factor that is cytotoxic to the respiratory epithelium causing direct lung injury allowing bacterial spread. Additionally it inhibits normal immune responses, for example by preventing dendritic cell maturation and therefore recruitment of adaptive immunity (134,135), and promotes the formation of neutrophil-platelet aggregates that may potentially contribute to cardiovascular complications in individuals with *S. pneumoniae* CAP (34). In preclinical mouse models of pneumococcal pneumonia two strategies have been demonstrated to be beneficial. Administration of neutralising monoclonal antibodies prior to infection with *S. pneumoniae* succeeded in increasing survival time and bacterial clearance from the lung and reducing bacteremia and histological evidence of lung injury (136), whilst a detoxified pneumolysin derivative antigen successfully induced neutralising antibodies that decreased the inflammatory response and lung injury associated with *S. pneumoniae* infection (137).

Neutrophil elastase inhibitors

Neutrophil elastases are released on degranulation, degrading phagocytosed proteins. However during infection normal regulation by proteases including alpha-1 antitrypsin can become overwhelmed allowing neutrophil elastases to promulgate lung injury by direct epithelial cell cytotoxicity in addition to impacting on both destruction and accumulation of the extracellular matrix, as well as prolonging the inflammatory response (138). Additionally there is evidence that neutrophil elastase activity can persist even after clinical improvement from an infection (139) and can drive the pathogenesis of both emphysema and pulmonary fibrosis post-infection (140).

In Japan, sivelestat, a neutrophil elastase inhibitor, is in clinical use for pneumonia-associated ARDS following an initial phase III trial that suggested benefit in these patients (141). However subsequently STRIVE, a multinational RCT, showed no improvement in mortality, ventilator-free days or pulmonary function in mechanically ventilated patients with acute lung injury of any aetiology

given sivelestat (142). The trial was discontinued prematurely in view of a trend of increased long-term mortality in the sivelestat arm. A number of studies since have also failed to demonstrate tangible benefit with a meta-analysis of 8 trials of sivelestat for ARDS of varying aetiologies showing no reduction in 28- to 30-day mortality including in subgroup analysis of only Japanese studies, a suggestion of decreased 180-day mortality in the placebo arms of borderline statistical significance, no impact on ventilator-free days, and only a minor improvement in paO2:FiO2 at day 3 (143). A recent Japanese retrospective observational study of severe pneumonia patients also showed no difference in 7- or 30-day mortality in patients receiving sivelestat in propensity matched groups. Due to the lack of benefit identified in the latest studies sivelestat is unlikely to be used in patients with severe pneumonia.

Summary

As demonstrated by Austrian and Gold, the single best intervention in terms of reducing mortality in pneumonia is effective antimicrobial therapy and therefore timely and appropriate microbiological sampling and antimicrobial administration will continue to be of the essence. However there remains a high mortality in patients with severe CAP despite antimicrobial administration mandating the search for adjunctive immunomodulatory therapies for CAP and the associated sequelae sepsis and ARDS. Despite much promise in pre-clinical work, many clinical trials have unfortunately failed to demonstrate a benefit and there are a number of areas where the data has been conflicting, for example PROWESS versus PROWESS-SHOCK, and CAPTIVATE versus phase II rTFPI data. Current evidence supports the use of macrolide combination antibiotic therapy and unless contraindicated continuation of pre-admission statin and antiplatelet therapy, and suggests it may be reasonable to consider low dose corticosteroids for a minimum of 7 days following an initial bolus on an individual basis in patients with severe CAP and a hyperinflammatory phenotype given the potential for improving patient-centered and economic outcomes with negligible adverse effects. Additionally, a growing body of available evidence does not support the use of drugs such as sivelestat. Results of RCTs evaluating aspirin, IVIg and thrombomodulin are awaited and may yet influence practice, whilst there are other areas deserving of similar trials. Further away, there are a number of significant causes for optimism in preclinical studies, which need to be developed to permit clinical trials.

Moving forwards, in addition to pursuing these potential therapeutic targets for immunomodulation, there are a number of areas worthy of study. Firstly, current determination of CAP severity is based on crude scoring systems or criteria, whereas to derive optimal benefit from immunomodulatory strategies used in clinical practice to date we need to be able to reliably identify patients with or at risk of developing significant immune dysregulation, which requires adequately evaluated biomarkers. This both enables identification of patients likely to benefit from a therapy, but also facilitates more homogenous trial populations increasing the likelihood of positive outcomes. Secondly, nebulized and inhaled routes of administration enable delivery of the drug to the intended site of action with the potential to reduce systemic adverse effects and may well be utilized for a growing number of these adjunctive therapies. Thirdly, as we understand more about the pathogenesis of specific infections, it may be that the optimum strategy for adjunctive therapies differs with each, making early identification of the causative pathogen of even greater importance. Finally, for a number of these interventions, timing appears to be crucial, reflecting the evolution of inflammatory responses over the course of infection and the challenges in reversing inflammation and tissue damage. This means that rather than adjunctive treatments, a number of these therapies are best placed as prophylactic measures given during identified high risk periods to high risk individuals.

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Footnote

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References

1. José RJ, Periselneris JN, Brown JS. Community-acquired pneumonia. Curr Opin Pulm Med 2015;21:212-8.
2. Torres A, Peetermans WE, Viegi G, et al. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013;68:1057-65.
3. British Lung Foundation. (2017). Pneumonia statistics. Available online: https://statistics.blf.org.uk/pneumonia

4. National Institute for Health and Clinical Evidence. NICE clinical guideline: Pneumonia Scope. 2012. Available online: https://www.nice.org.uk/guidance/cg191/documents/pneumonia-final-scope2

5. Secondary Care Analysis Team (NHS Digital), Winter J. Hospital Admitted Patient Care Activity: 2015-16. 2016. Available online: http://content.digital.nhs.uk/catalogue/PUB22378/hosp-epis-stat-admi-summ-rep-2015-16-rep.pdf

6. Rui P, Kang K, Albert M. National Hospital Ambulatory Medical Care Survey: 2013 Emergency Department Summary Tables. Available online: http://www.cdc.gov/nchs/data/ahcd/nhamcs_emergency/2013_ed_web_tables.pdf

7. Anon. Deaths Registered in England and Wales (Series DR), 2013 Key Findings. 2014. Available online: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/deathsregistereingenglandandwalesseriesdr/previousReleases

8. Kochanek KD, Murphy SL, Xu J, et al. National Vital Statistics Reports, Volume 65, Number 4, (06/30/2016). 2014. Available online: https://www.cdc.gov/nchs/data/nvsr/nvsr65/nvsr65_04.pdf

9. Austrian R, Gold J. Pneumococcal Bacteraemia with Especial Reference to Bacteremic Pneumococcal Pneumonia. Ann Intern Med 1964;60:759.

10. Feikin DR, Schuchat A, Kolczak M, et al. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995-1997. Am J Public Health 2000;90:223-9.

11. Restrepo MI, Anzueto A. Severe Community-Acquired Pneumonia. Infect Dis Clin North Am 2009;23:503-20.

12. José RJ, Brown JS. Predicting bacteraemia or rapid identification of the causative pathogen in community acquired pneumonia: where should the priority lie? Eur Respir J 2016;48:619-22.

13. Woodhead M. Community-acquired pneumonia in Europe: causative pathogens and resistance patterns. Eur Respir J Suppl 2002;36:20s-7s.

14. Feldman C. Clinical relevance of antimicrobial resistance in the management of pneumococcal community-acquired pneumonia. J Lab Clin Med 2004;143:269-83.

15. Bordon J, Aliberti S, Fernandez-Botran R, et al. Understanding the roles of cytokines and neutrophil activity and neutrophil apoptosis in the protective versus deleterious inflammatory response in pneumonia. Int J Infect Dis 2013;17:e76-83.

16. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801.

17. Lee WL, Downey GP. Neutrophil activation and acute lung injury. Curr Opin Crit Care 2001;7:1-7.

18. Summers C, Singh NR, White JF, et al. Pulmonary retention of primed neutrophils: a novel protective host response, which is impaired in the acute respiratory distress syndrome. Thorax 2014;69:623-9.

19. Levi M, ten Cate H. Disseminated Intravascular Coagulation. N Engl J Med 1999;341:586-92.

20. Eisele NA, Anderson DM. Host Defense and the Airway Epithelium: Frontline Responses That Protect against Bacterial Invasion and Pneumonia. J Pathog 2011;2011:249802.

21. Martin TR, Frevert CW. Innate immunity in the lungs. Proc Am Thorac Soc 2005;2:403-11.

22. Eddens T, Kolls JK. Host defenses against bacterial lower respiratory tract infection. Curr Opin Immunol 2012;24:424-30.

23. Periselneris J, José RJ, Brown J, et al. Targeting Inflammatory Responses to Streptococcus pneumoniae. New Horizons Transl Med 2015;2:167-74.

24. Camberlein E, Cohen JM, José R, et al. Importance of bacterial replication and alveolar macrophage-independent clearance mechanisms during early lung infection with Streptococcus pneumoniae. Infect Immun 2015;83:1181-9.

25. Chalmers J, Pletz M, Aliberti S. European Respiratory Monograph 63: Community-Acquired Pneumonia. Welte T. editor. Sheffield: European Respiratory Society, 2014.

26. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013;13:159-75.

27. Remick DG, Bolgos G, Copeland S, et al. Role of interleukin-6 in mortality from and physiologic response to sepsis. Infect Immun 2005;73:2751-7.

28. Martínez R, Menéndez R, Reyes S, et al. Factors associated with inflammatory cytokine patterns in community-acquired pneumonia. Eur Respir J 2011;37:393-9.

29. Fernandez-Botran R, Uriarte SM, Arnold FW, et al. Contrasting Inflammatory Responses in Severe and Non-severe Community-acquired Pneumonia. Inflammation 2014;37:1158-66.

30. José RJ, Williams AE, Chambers RC. Proteinase-activated receptors in fibroproliferative lung disease. Thorax 2014;69:190-2.

31. Asaduzzaman M, Rahman M, Jeppsson B, et al. P-selectin glycoprotein-ligand-1 regulates pulmonary recruitment of neutrophils in a platelet-independent manner in abdominal sepsis. Br J Pharmacol 2009;156:307-15.

32. Osterud B. Tissue factor expression by monocytes: regulation and pathophysiological roles. Blood Coagul Fibrinolysis 1998;9 Suppl 1:S9-14.

33. José RJ, Williams AE, Mercer PF, et al. Regulation of neutrophilic inflammation by proteinase-activated receptor 1 during bacterial pulmonary infection. J Immunol 2015;194:6024-34.

34. Nel JG, Durandt C, Theron AJ, et al. Pneumolysin mediates heterotypic aggregation of neutrophils and platelets in vitro. J Infect 2017;74:599-608.

35. van der Poll T, de Boer JD, Levi M. The effect of inflammation on coagulation and vice versa. Curr Opin Infect Dis 2011;24:273-8.

36. Demetz G, Ott I. The interface between inflammation and coagulation in cardiovascular disease. Int J Inflam 2012;2012:860301.

37. Levi M, ten Cate H, van der Poll T. Endothelium: interface between coagulation and inflammation. Crit Care Med 2002;30:S220-4.

38. Levi M, Van Der Poll T, Büller HR. Bidirectional relation between inflammation and coagulation. Circulation 2004;109:2698-704.

39. Altenburg J, de Graaff CS, van der Werf TS, et al. Immunomodulatory Effects of Macrolide Antibiotics - Part 1: Biological Mechanisms. Respiration 2011;81:67-74.

40. Kudoh S, Uetake T, Hagiwara K, et al. Clinical effects of low-dose long-term erythromycin chemotherapy on diffuse panbronchiolitis. Nihon Kyobu Shikkan Gakkai Zasshi 1987;25:632-42.

41. Sligl WI, Asadi L, Eurich DT, et al. Macrolides and Mortality in Critically Ill Patients With Community-Acquired Pneumonia: A Systematic Review and Meta-Analysis. Crit Care Med 2014;42:420-32.

42. Restrepo MI, Mortensen EM, Waterer GW, et al. Impact of macrolide therapy on mortality for patients with severe sepsis due to pneumonia. Eur Respir J 2009;33:153-9.

43. Arnold FW, Wiemken TL, Peyrani P, et al. Mortality differences among hospitalized patients with community-acquired pneumonia in three world regions: Results from the Community-Acquired Pneumonia Organization (CAPO) International Cohort Study. Respir Med 2013;107:1101-11.

44. Postma DF, van Werkhoven CH, van Elden LJ, et al. Antibiotic Treatment Strategies for Community-Acquired Pneumonia in Adults. N Engl J Med 2015;372:1312-23.

45. Salluh JI, Verdeal JC, Mello GW, et al. Cortisol levels in patients with severe community-acquired pneumonia. Intensive Care Med 2006;32:595-8.

46. Annane D, Sébille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002;288:862-71.

47. Chen Y, Li K, Pu H, et al. Corticosteroids for pneumonia. Cochrane Database Syst Rev 2011;(3):CD007720.

48. Nie W, Zhang Y, Cheng J, et al. Corticosteroids in the treatment of community-acquired pneumonia in adults: a meta-analysis. PLoS One 2012;7:e47926.

49. Confalonieri M, Urbino R, Potena A, et al. Hydrocortisone Infusion for Severe Community-acquired Pneumonia. Am J Respir Crit Care Med 2005;171:242-8.

50. Torres A, Sibila O, Ferrer M, et al. Effect of Corticosteroids on Treatment Failure Among Hospitalized Patients With Severe Community-Acquired Pneumonia and High Inflammatory Response. JAMA 2015;313:677.

51. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2015;385:1511-8.

52. Wan YD, Sun TW, Liu ZQ, et al. Efficacy and Safety of Corticosteroids for Community-Acquired Pneumonia. Chest 2016;149:209-19.

53. Tagami T, Matsui H, Horiguchi H, et al. Low-dose corticosteroid use and mortality in severe community-acquired pneumonia patients. Eur Respir J 2015;45:463-72.

54. Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. N Engl J Med 2008;358:111-24.

55. Blanco-Colio LM, Tuñón J, Martín-Ventura JL, et al. Anti-inflammatory and immunomodulatory effects of statins. Kidney Int 2003;63:12-23.

56. Troeman DPR, Postma DF, van Werkhoven CH, et al. The immunomodulatory effects of statins in community-acquired pneumonia: A systematic review. J Infect 2013;67:93-101.

57. Novack V, Eisinger M, Frenkel A, et al. The effects of statin therapy on inflammatory cytokines in patients with bacterial infections: A randomized double-blind placebo controlled clinical trial. Intensive Care Med 2009;35:1255-60.

58. Myles PR, Hubbard RB, McKeever TM, et al. Risk of community-acquired pneumonia and the use of statins, ace inhibitors and gastric acid suppressants: A population-based case-control study. Pharmacoepidemiol Drug Saf 2009;18:269-75.

59. Mortensen EM, Restrepo MI, Anzueto A, et al. The

effect of prior statin use on 30-day mortality for patients hospitalized with community-acquired pneumonia. Respir Res 2005;6:82.

60. Dublin S, Jackson ML, Nelson JC, et al. Statin use and risk of community acquired pneumonia in older people: population based case-control study. BMJ 2009;338:b2137.

61. Rothenberg FG, Clay MB, Jamali H, et al. Systematic review of β blocker, aspirin, and statin in critically ill patients: importance of severity of illness and cardiac troponin. J Investig Med 2017;65:747-53.

62. Hackam DG, Mamdani M, Li P, et al. Statins and sepsis in patients with cardiovascular disease: A population-based cohort analysis. Lancet 2006;367:413-8.

63. Wootton DG, Diggle PJ, Court J, et al. Recovery from pneumonia requires efferocytosis which is impaired in smokers and those with low body mass index and enhanced by statins. Thorax 2016;71:1052-4.

64. Sapey E, Patel JM, Greenwood HL, et al. Pulmonary Infections in the Elderly Lead to Impaired Neutrophil Targeting, Improved by Simvastatin. Am J Respir Crit Care Med 2017. [Epub ahead of print].

65. Thomas G, Hraiech S, Loundou A, et al. Statin therapy in critically-ill patients with severe sepsis: a review and meta-analysis of randomized clinical trials. Minerva Anestesiol 2015;81:921-30.

66. Kruger P, Bailey M, Bellomo R, et al. A multicenter randomized trial of atorvastatin therapy in intensive care patients with severe sepsis. Am J Respir Crit Care Med 2013;187:743-50.

67. McAuley DF, Laffey JG, O'Kane CM, et al. Simvastatin in the Acute Respiratory Distress Syndrome. N Engl J Med 2014;371:1695-703.

68. Farkhutdinov U, Mirchaidarov A, Farkhutdinov S. Intravenous immunoglobulin in community acquired pneumonia. Eur Respir J 2011;38:818.

69. de la Torre MC, Torán P, Serra-Prat M, et al. Serum levels of immunoglobulins and severity of community-acquired pneumonia. BMJ Open Respir Res 2016;3:e000152.

70. Herer B, Labrousse F, Mordelet-Dambrine M, et al. Selective IgG subclass deficiencies and antibody responses to pneumococcal capsular polysaccharide antigen in adult community-acquired pneumonia. Am Rev Respir Dis 1990;142:854-7.

71. Welte T, Dellinger RP, Ebelt H, et al. Concept for a study design in patients with severe community-acquired pneumonia: A randomised controlled trial with a novel IGM-enriched immunoglobulin preparation - The CIGMA study. Respir Med 2015;109:758-67.

72. Mouthon L, Kaveri SV, Spalter SH, et al. Mechanisms of action of intravenous immune globulin in immune-mediated diseases. Clin Exp Immunol 1996;104 Suppl 1:3-9.

73. Kreymann KG, de Heer G, Nierhaus A, et al. Use of polyclonal immunoglobulins as adjunctive therapy for sepsis or septic shock. Crit Care Med 2007;35:2677-85.

74. Werdan K, Pilz G, Bujdoso O, et al. Score-based immunoglobulin G therapy of patients with sepsis: The SBITS study. Crit Care Med 2007;35:2693-701.

75. Laupland KB, Kirkpatrick AW, Delaney A. Polyclonal intravenous immunoglobulin for the treatment of severe sepsis and septic shock in critically ill adults: A systematic review and meta-analysis. Crit Care Med 2007;35:2686-92.

76. Alejandria MM, Lansang MA, Dans LF, et al. Intravenous immunoglobulin for treating sepsis, severe sepsis and septic shock. Cochrane Database Syst Rev 2013:CD001090.

77. Tagami T, Matsui H, Fushimi K, et al. Intravenous Immunoglobulin and Mortality in Pneumonia Patients with Septic Shock: An Observational Nationwide Study. Clin Infect Dis 2015;61:385-92.

78. Berlot G, Vassallo MC, Busetto N, et al. Relationship between the timing of administration of IgM and IgA enriched immunoglobulins in patients with severe sepsis and septic shock and the outcome: A retrospective analysis. J Crit Care 2012;27:167-71.

79. Tuinman PR, Dixon B, Levi M, et al. Nebulized anticoagulants for acute lung injury - a systematic review of pre-clinical and clinical investigations. Crit Care 2012;16:R70.

80. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and Safety of Recombinant Human Activated Protein C for Severe Sepsis. N Engl J Med 2001;344:699-709.

81. Finfer S, Ranieri VM, Thompson BT, et al. Design, conduct, analysis and reporting of a multi-national placebo-controlled trial of activated protein C for persistent septic shock. Intensive Care Med 2008;34:1935-47.

82. Dellinger RP, Carlet JM, Masur H, et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Intensive Care Med 2004;30:536-55.

83. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 2008;34:17-60.

84. Abraham E, Laterre PF, Garg R, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. N Engl J Med 2005;353:1332-41.

85. Vincent JL, Bernard GR, Beale R, et al. Drotrecogin alfa (activated) treatment in severe sepsis from the global open-label trial ENHANCE: Further evidence for survival and safety and implications for early treatment. Crit Care Med 2005;33:2266-77.

86. Ranieri VM, Thompson BT, Barie PS, et al. Drotrecogin Alfa (Activated) in Adults with Septic Shock. N Engl J Med 2012;366:2055-64.

87. Laterre PF, Garber G, Levy H, et al. Severe community-acquired pneumonia as a cause of severe sepsis: Data from the PROWESS study. Crit Care Med 2005;33:952-61.

88. Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. JAMA 2003;290:238-47.

89. LaRosa SP, Opal SM. Tissue factor pathway inhibitor and antithrombin trial results. Crit Care Clin 2005;21:433-48.

90. Wunderink RG, Laterre PF, Francois B, et al. Recombinant Tissue Factor Pathway Inhibitor in Severe Community-acquired Pneumonia. Am J Respir Crit Care Med 2011;183:1561-8.

91. Baudo F, Caimi TM, de Cataldo F, et al. Antithrombin III (ATIII) replacement therapy in patients with sepsis and/or postsurgical complications: a controlled double-blind, randomized, multicenter study. Intensive Care Med 1998;24:336-42.

92. Warren BL, Eid A, Singer P, et al. High-Dose Antithrombin III in Severe Sepsis. JAMA 2001;286:1869-78.

93. Allingstrup M, Wetterslev J, Ravn FB, et al. Antithrombin III for critically ill patients. Cochrane Database Syst Rev 2016;2:CD005370.

94. Tagami T, Matsui H, Horiguchi H, et al. Antithrombin and mortality in severe pneumonia patients with sepsis-associated disseminated intravascular coagulation: an observational nationwide study. J Thromb Haemost 2014;12:1470-9.

95. Vincent JL, Ramesh MK, Ernest D, et al. A Randomized, Double-Blind, Placebo-Controlled, Phase 2b Study to Evaluate the Safety and Efficacy of Recombinant Human Soluble Thrombomodulin, ART-123, in Patients With Sepsis and Suspected Disseminated Intravascular Coagulation. Crit Care Med 2013;41:2069-79.

96. Hata A, Kobayashi Y, Toyonaga Y, et al. Two cases of acute exacerbation of interstitial pneumonia treating with recombinant thrombomodulin: 12AP3 5. Eur J Anaesthesiol 2014;31:202.

97. Abe M, Tsushima K, Matsumura T, et al. Efficacy of thrombomodulin for acute exacerbation of idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia: A nonrandomized prospective study. Drug Des Devel Ther 2015;9:5755-62.

98. Isshiki T, Sakamoto S, Kinoshita A, et al. Recombinant human soluble thrombomodulin treatment for acute exacerbation of idiopathic pulmonary fibrosis: A retrospective study. Respiration 2015;89:201-7.

99. Tagami T, Matsui H, Horiguchi H, et al. Recombinant human soluble thrombomodulin and mortality in severe pneumonia patients with sepsis-associated disseminated intravascular coagulation: An observational nationwide study. J Thromb Haemost 2015;13:31-40.

100. Asahi Kasei Pharma America Corporation. Phase 3 Safety and Efficacy Study of ART-123 in Subjects With Severe Sepsis and Coagulopathy. Available online: https://clinicaltrials.gov/ct2/show/NCT01598831

101. Weber C, Erl W, Pietsch A, et al. Aspirin inhibits nuclear factor-kappa B mobilization and monocyte adhesion in stimulated human endothelial cells. Circulation 1995;91:1914-7.

102. Taubert D, Berkels R, Grosser N, et al. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol 2004;143:159-65.

103. El Kebir D, József L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med 2009;180:311-9.

104. Falcone M, Russo A, Cangemi R, et al. Lower Mortality Rate in Elderly Patients With Community-Onset Pneumonia on Treatment With Aspirin. J Am Heart Assoc 2015;4:e001595.

105. Boyle AJ, Di Gangi S, Hamid UI, et al. Aspirin therapy in patients with acute respiratory distress syndrome (ARDS) is associated with reduced intensive care unit mortality: a prospective analysis. Crit Care 2015;19:109.

106. Mohananey D, Sethi J, Villablanca PA, et al. Effect of antiplatelet therapy on mortality and acute lung injury in critically ill patients: A systematic review and meta-analysis. Ann Card Anaesth 2016;19:626-37.

107. Wang L, Li H, Gu X, et al. Effect of antiplatelet therapy on acute respiratory distress syndrome and mortality in critically ill patients: A meta-analysis. PLoS One 2016;11:e0154754.

108. Kor DJ, Talmor DS, Banner-Goodspeed VM, et al. Lung Injury Prevention with Aspirin (LIPS-A): a protocol for a multicentre randomised clinical trial in medical patients at high risk of acute lung injury. BMJ Open 2012;2(5).

109. McAuley D. ASpirin as a Treatment for ARDS (STAR): a Phase 2 Randomised Control Trial. Available online: https://clinicaltrials.gov/ct2/show/NCT02326350

110. McAuley D. The Effect of Aspirin on REducing iNflammation in Human in Vivo Model of Acute Lung Injury (ARENA). Available online: https://clinicaltrials.gov/ct2/show/NCT01659307

111. Bernard G. Ticagrelor in Severe Community Acquired Pneumonia. Available online: https://clinicaltrials.gov/ct2/show/NCT01998399

112. Mifsud EJ, Tan AC, Jackson DC. TLR agonists as modulators of the innate immune response and their potential as agents against infectious disease. Front Immunol 2014;5:79.

113. O'Neill LA, Bryant CE, Doyle SL. Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer. Pharmacol Rev 2009;61:177-97.

114. Tomlinson G, Chimalapati S, Pollard T, et al. TLR-mediated inflammatory responses to Streptococcus pneumoniae are highly dependent on surface expression of bacterial lipoproteins. J Immunol 2014;193:3736-45.

115. Lima CX, Souza DG, Amaral FA, et al. Therapeutic effects of treatment with anti-TLR2 and anti-TLR4 monoclonal antibodies in polymicrobial sepsis. PLoS One 2015;10:e0132336.

116. Opal SM, Laterre PF, Francois B, et al. Effect of Eritoran, an Antagonist of MD2-TLR4, on Mortality in Patients With Severe Sepsis. JAMA 2013;309:1154.

117. Lynn M, Rossignol DP, Wheeler JL, et al. Blocking of Responses to Endotoxin by E5564 in Healthy Volunteers with Experimental Endotoxaemia. J Infect Dis 2003;187:631-9.

118. Reppe K, Tschernig T, Luhrmann A, et al. Immunostimulation with macrophage-activating lipopeptide-2 increased survival in murine pneumonia. Am J Respir Cell Mol Biol 2009;40:474-81.

119. Abdul-Careem MF, Firoz Mian M, Gillgrass AE, et al. FimH, a TLR4 ligand, induces innate antiviral responses in the lung leading to protection against lethal influenza infection in mice. Antiviral Res 2011;92:346-55.

120. Yu FS, Cornicelli MD, Kovach MA, et al. Flagellin Stimulates Protective Lung Mucosal Immunity: Role of Cathelicidin-Related Antimicrobial Peptide. J Immunol 2010;185:1142-9.

121. Muñoz N, Van Maele L, Marqués JM, et al. Mucosal administration of flagellin protects mice from Streptococcus pneumoniae lung infection. Infect Immun 2010;78:4226-33.

122. Porte R, Fougeron D, Muñoz-Wolf N, et al. A toll-like receptor 5 agonist improves the efficacy of antibiotics in treatment of primary and influenza virus-associated pneumococcal mouse infections. Antimicrob Agents Chemother 2015;59:6064-72.

123. Duggan JM, You D, Cleaver JO, et al. Synergistic Interactions of TLR2/6 and TLR9 Induce a High Level of Resistance to Lung Infection in Mice. J Immunol 2011;186:5916-26.

124. Tuvim MJ, Evans SE, Clement CG, et al. Augmented lung inflammation protects against influenza A pneumonia. PLoS One 2009;4:e4176.

125. Leiva-Juárez MM, Ware HH, Kulkarni VV, et al. Inducible epithelial resistance protects mice against leukaemia-associated pneumonia. Blood 2016;128:982-92.

126. Schouten M, van't Veer C, Roelofs JJ, et al. Protease-activated receptor-1 impairs host defense in murine pneumococcal pneumonia: a controlled laboratory study. Crit Care 2012;16:R238.

127. Kaneider NC, Leger AJ, Agarwal A, et al. "Role reversal" for the receptor PAR1 in sepsis-induced vascular damage. Nat Immunol 2007;8:1303-12.

128. Chatterjee S, Sharma A, Mukherjee D. PAR-1 antagonists: Current state of evidence. J Thromb Thrombolysis 2013;35:1-9.

129. Gao F, Chiu SM, Motan DAL, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 2016;7:e2062.

130. Vasandan AB, Jahnavi S, Shashank C, et al. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE2-dependent mechanism. Sci Rep 2016;6:38308.

131. Alcayaga-Miranda F, Cuenca J, Khoury M. Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. Front Immunol 2017;8:339.

132. Lombardo E. Mesenchymal stem cells as a therapeutic tool to treat sepsis. World J Stem Cells 2015;7:368.

133. TiGenix S.A.U. (2017). Sepcell stem cell therapy for community acquired pneumonia induced sepsis. Available online: http://www.sepcell.eu/

134. Rubins JB, Janoff EN. Pneumolysin: a multifunctional pneumococcal virulence factor. J Lab Clin Med 1998;131:21-7.

135. Littmann M, Albiger B, Frentzen A, et al. Streptococcus pneumoniae evades human dendritic cell surveillance by pneumolysin expression. EMBO Mol Med 2009;1:211-22.

136. García-Suárez Mdel M, Cima-Cabal MD, Flórez N, et

al. Protection against pneumococcal pneumonia in mice by monoclonal antibodies to pneumolysin. Infect Immun 2004;72:4534-40.

137. Kang J, Bezerra B de B, Pacios S, et al. Aggregatibacter actinomycetemcomitans infection enhances apoptosis in vivo through a caspase-3-dependent mechanism in experimental periodontitis. Infect Immun 2012;80:2247-56.

138. Greene C, Taggart C, Lowe G, et al. Local impairment of anti-neutrophil elastase capacity in community-acquired pneumonia. J Infect Dis 2003;188:769-76.

139. Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. Thorax 2009;64:81-8.

140. Chua F. Neutrophil Elastase: Mediator of Extracellular Matrix Destruction and Accumulation. Proc Am Thorac Soc 2006;3:424-7.

141. Tamakuma S, Shiba T, Hirasawa H, et al. A phase III clinical study of neutrophil elastase inhibitor ONO-5046 Na in SIRS patients. J Clin Ther Med 1998;14:289-318.

142. Zeiher BG, Artigas A, Vincent J, et al. Neutrophil elastase inhibition in acute lung injury: Results of the STRIVE study. Crit Care Med 2004;32:1695-702.

143. Iwata K, Doi A, Ohji G, et al. Effect of Neutrophil Elastase Inhibitor (Sivelestat Sodium) in the Treatment of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS): A Systematic Review and Meta-Analysis. Intern Med 2010;49:2423-32.