Pathogenesis, treatment, and prevention of pneumococcal pneumonia

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Pneumococcus remains the most common cause of community-acquired pneumonia worldwide. *Streptococcus pneumoniae* is well adapted to people, and is a frequent inhabitant of the upper airways in healthy hosts. This seemingly innocuous state of colonisation is a dynamic and competitive process in which the pathogen attempts to engage the host, proliferate, and invade the lower airways. The host in turn continuously deploys an array of innate and acquired cellular and humoral defences to prevent pneumococci from breaching tissue barriers. Discoveries into essential molecular mechanisms used by pneumococci to evade host-sensing systems that are designed to contain the pathogen provide new insights into potential treatment options. Versatility of the genome of pneumococci and the bacteria's polygenic virulence capabilities show that a multifaceted approach with many vaccine antigens, antibiotic combinations, and immunoadjuvant therapies will be needed to control this microbe.

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Introduction

Nearly a century ago, Sir William Osler proclaimed *Streptococcus pneumoniae* (or pneumococcus) as "the captain of all the men of death".[1] This statement remains as true today as it was then. Severe community-acquired pneumonia is the most common cause of death from infection in developed countries, and the pneumococcus is the most frequent cause of lower respiratory tract infection. We can now control many other respiratory pathogens of man reasonably well (eg, influenza, pertussis, tuberculosis, and *Haemophilus influenzae*), yet pneumococcus remains the main cause of community-acquired pneumonia worldwide.[1]

Reasons for the success of this obligate human pathogen are becoming increasingly apparent as the basic pathogenic mechanisms of pneumococcal pneumonia are discovered. This pathogen causes at least 1·2 million infant deaths every year worldwide.[2,3] The yearly death toll attributable to pneumococci in patients with AIDS and other immunocompromised states, elderly people, and those with comorbid illnesses is difficult to quantify, but probably exceeds the infant mortality rate.

Advances in comparative genomics and insights into innate and acquired immune-signalling mechanisms could provide new treatment options for pneumococcal disease.[2–8] We focus on the clinical outcomes after the initial host–pathogen interaction when *S pneumoniae* first reaches the airways and invades the lower respiratory tract.

Epidemiology

S pneumoniae is a common inhabitant of the upper respiratory tract, existing mainly as a commensal bacterium along with other co-resident microorganisms identified on the respiratory epithelium. After colonisation by one of 91 presently recognised serotypes, a new strain eliminates other competing pneumococcal serotypes, and persists for weeks (in adults) or months (in children), usually without any adverse sequelae. This carrier state maintains the organism within human populations, and induces some acquired B-cell-mediated immunity to reinfection.[3,4]

Genetic and epidemiological evidence show that one of two survival strategies account for the success of *S pneumoniae*. Specific clones are selected with either an invasive pneumococcal disease phenotype or a persistent colonisation phenotype with low risk of tissue invasion. Success of the phenotype of invasive pneumococcal disease depends on its capacity for rapid disease induction and efficient person-to-person spread by coughing. By contrast, the non-invasive phenotype uses various surface adhesins, immune evasion strategies, and secretory defences such as IgA1 protease and inhibitors of antibacterial peptides to help with long-term carriage within the nasopharynx.[5] Persistent carriage of pneumococci allows for low-level and longlasting transmission, thereby retaining non-invasive strains in human populations. Defects in host defences can alter this host–pathogen interaction and allow strains of low virulence to invade the immunocompromised host.[5–7]

The pneumococcus is mainly transmitted by direct contact with contaminated respiratory secretions between household members, infants, and children. Pneumococci are generally not regarded as highly contagious, and respiratory isolation of patients who are infected in the community or hospital settings is rarely indicated. However, a large, community-wide outbreak of serotype 5 pneumococci in Vancouver, Canada,[9] showed that potential exists for epidemic spread of *S pneumoniae*

Search strategy and selection criteria

We searched Medline and PubMed in English with the search terms "pneumococci", "*Streptococcus pneumoniae*", "pneumococcal pneumonia", "community acquired pneumonia", and "pneumococcal pathogenesis" for reports relating to pneumococcal pneumonia published in the past 10 years until January, 2009. We reviewed the publications and searched the reference lists of identified articles for older reports we judged to be of major importance.

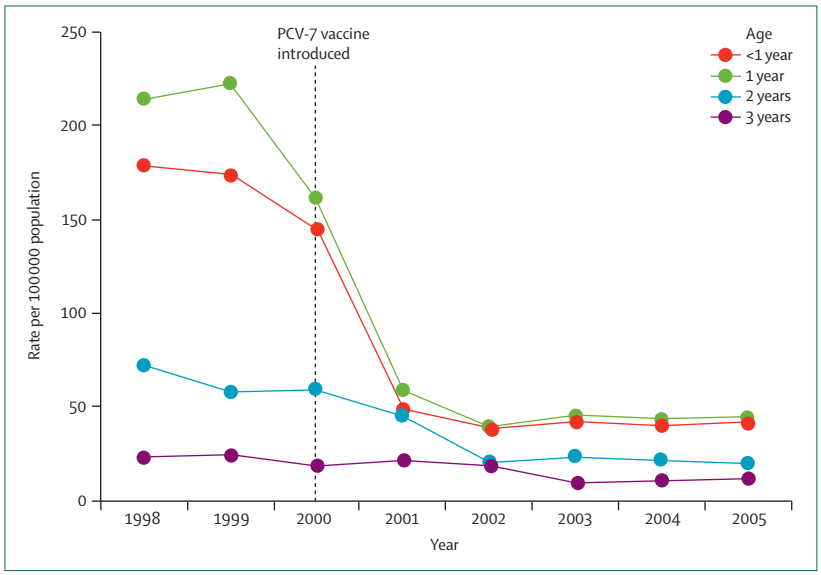

Figure 1: **Incidence of pneumococcal disease in children in the USA before and after introduction of the PCV-7 vaccine**
PCV-7=conjugate seven-valent pneumococcal vaccine. Reprinted with permission.[17]

within urban neighbourhoods. Similar small-scale outbreaks have been reported in day-care centres, jails, military bases, and men's shelters.[10–12] Airway colonisation by pneumococci is readily detectable in about 10% of healthy adults. 20–40% of healthy children are carriers, and more than 60% of infants and children in day-care settings can be carriers.[3,8]

Most people colonised with *S pneumoniae* have only one serotype at a time, although simultaneous carriage of more than one serotype is possible.[13] Duration of pneumococcal colonisation in an individual is highly serotype-specific.[14] Recent acquisition of an invasive serotype is more important as a determinant of subsequent risk of invasive pneumococcal disease than is duration of colonisation or specific clonal genotype.[15] Results of studies of the conjugate seven-valent pneumococcal vaccine in children suggest that the humoral immune response can reduce risk of serotype-specific disease, decrease likelihood of transmission in vaccine recipients, and even reduce transmission risk between non-immunised siblings and adults (ie, herd immunity).[3,16]

Incidence of invasive pneumococcal disease (ie, bacterial pneumonia and bacteraemia) varies substantially by age, genetic background, socioeconomic status, immune status, and geographical location. In a large US epidemiological survey in 1999, overall incidence in children older than 5 years was 98·7 per 100 000 per year. By 2005, the same survey showed that incidence had reduced by 75% to 23·4 per 100 000 per year. This change was largely attributable to reduced rates of infection with serotypes contained in the pneumococcal conjugate vaccine[17] (figure 1). This decreased overall incidence was accompanied by a small rise in invasive pneumococcal disease from non-vaccine serotypes.[18] This pattern seems to be continuing and has prompted a renewed interest in other vaccine formulations. Incidence rates can be as high as 441 cases per 100 000 per year in isolated Indigenous populations such as Alaskan Eskimos, Aboriginal Australians, and the Maoris of New Zealand.[19]

Other recognised risk factors for invasive pneumococcal disease in children and adults include asplenia, alcoholism, diabetes mellitus, age greater than 65 years, underlying lung disease, severe liver disease, influenza and probably other respiratory viruses, immunoglobulin and complement deficiencies, and other immunocompromised states, including HIV infection. Crowding, recent acquisition of a virulent strain, poverty, cigarette smoking, proton pump inhibitors,[20] and other risk factors probably contribute to pneumococcal disease (panel).[3,14] Antecedent respiratory infections and recent exposure to antibiotics seem to be additional risk factors for colonisation of airways and invasive pneumococcal disease. Antibiotic-induced alterations in competing inhibitory bacteria (α-haemolytic streptococci in particular), release of viral enzymes (eg, influenza neuraminidase) that promote adherence,[3] and host inflammation-induced expression of pneumococcal invasion receptors on host cells, such as platelet-activating factor receptor[21] and CD14,[22] contribute to pneumococcal colonisation and invasion.

Genetics and virulence of *S pneumoniae*

Knowledge of genomes of many invasive and non-invasive strains of *S pneumoniae* enable detailed comparative analyses.[23–29] The pneumococcal genome has between 2 million and 2·1 million basepairs, dependent on strain virulence (figure 2). It is a covalently closed, circular DNA structure often accompanied by small cryptic plasmids. The guanine–cytosine content of *S pneumoniae* DNA (39·7%) is lower than that of many other bacterial pathogens. One of the original pneumococcal genomes to be sequenced, strain TIGR4,[23] has 2236 open reading frames of which two-thirds have assigned roles for their predicted gene products. Another 16% of open reading frames generate conserved, hypothetical proteins of unknown function. About 20% exist only in *S pneumoniae*.[23]

The genome contains a core set of 1553 genes that are essential for viability.[24–29] An additional 154 genes form the complement of bacterial genes that collectively contribute to virulence (the virulome), and 176 genes actively maintain a non-invasive phenotype. Pneumococci have an unusually large number of insertion sequences, accounting for up to 5% of the entire genome. Some strains contain conjugative transposons within their genomes that mediate antibiotic resistance. Much plasticity exists within the *S pneumoniae* genome, with up to 10% variation between strains. The genome is replete

Panel: Risk factors for pneumococcal pneumonia and invasive pneumococcal disease

Definite risk factors* (high risk)

- Younger than 2 years or older than 65 years
- Asplenia or hyposplenia
- Alcoholism
- Diabetes mellitus
- Antecedent influenza
- Defects in humoral immunity (complement or immunoglobulin)
- HIV infection
- Recent acquisition of a new virulent strain

Probable risk factors† (moderate risk)

- Genetic polymorphisms (eg, complement, MBL, IRAK-4, Mal, MyD88)
- Isolated populations
- Poverty, crowding, low pneumococcal vaccine use
- Cigarette smoking
- Chronic lung disease
- Severe liver disease
- Other antecedent viral infections
- Poor mucociliary function

Possible risk factors‡ (low risk)

- Recent exposure to antibiotics
- Defects in cellular immunity and neutrophil defects
- Diminished cough reflex, aspiration pneumonitis
- Proton-pump inhibitors and other gastric-acid inhibitors
- Large organism burden in upper airways
- Childhood day care

MBL=mannose binding lectin. IRAK=interleukin I receptor associated kinase. Mal=Myeloid differentiation primary response factor 88 adaptor like. *Many clinical studies. †Some clinical and laboratory studies. ‡Few clinical studies.

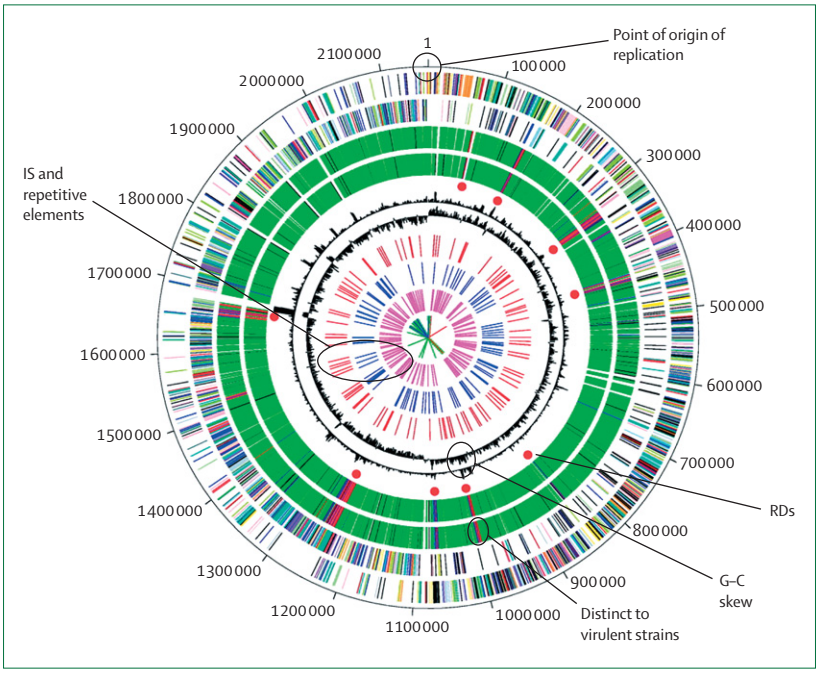

Figure 2: **Genomic structure of *Streptococcus pneumoniae* TIGR4**
RDs=regions of diversity. G–C skew=difference in guanine (G)–cytosine (C) ratio from G–C average of pneumococcal genome. IS=insertion sequence elements. Adapted from Tettelin, et al[23] with permission from the AAAS.

with many copies of direct-repeat DNA elements that provide recombination hotspots for genetic variability.[23]

Pneumococci express the most diverse array of substrate transport, and use systems known to human pathogens.[23,24] Specific ATP-binding cassette transporters import carbon or aminoacid substrates and export outer surface adhesins, degradation enzymes, or capsular synthetic components. These transporters are also essential for genetic competence (ability to take up homologous strands of naked DNA), for nutrient acquisition, and as efflux pumps to resist antibiotics.[23] Pneumococci have much genetic space for capsular polysaccharide synthesis—the most important virulence factor for this organism.[5] Phase variation arises with molecular switches at promoter regions of open reading frames for major surface antigens.[23] Pneumococci regulate the amount of capsular material produced during colonisation and invasion. Transparent (thin) capsules are favoured in early colonisation, whereas opaque (thick) capsules are favoured during invasion to resist complement-mediated opsonophagocytosis.[3,5]

Pneumococci cluster virulence genes into small defined sequences within the genome known as regions of diversity. These regions distinguish invasive from non-invasive strains. At least 13 such regions are identified in pneumococci, many of which carry the genetic signatures of pathogenicity islands.[25,28] Non-virulent strains exist even within the same capsular serotype that contain some but not all elements of the entire pneumococcal virulome.[5,29–31] Pneumococci have an array of two-component sensor-kinase signal systems that recognise environmental cues (eg, cell density, substrate availability, and microbial competitors) and alter their genetic programmes in response. These systems direct the synthesis of bactericins[32] (bacterial peptides that kill other related bacteria), competence genes for genetic recombination, biofilm formation,[33] and virulence expression.[5,33] Pneumococcal virulence has been acquired by horizontal transmission from other pathogens during the evolutionary past.[34] Evidence of continuing evolution through the acquisition of new antibiotic resistance genes and virulence traits is shown in pneumococci and other gram-positive bacterial pathogens, such as group A streptococci,[35] *Staphylococcus aureus*,[36] and *Clostridium difficile*.[37]

Pneumococcal virulence factors

Table 1 shows a summary of virulence traits identified in pneumococci.[38–49] An array of virulence factors needs to be expressed in a coordinated way for tissue invasion to be successful (phenotype for invasive pneumococcal disease). The most important virulence determinants

	Mechanism of action	Level of evidence*
Polysaccharide capsule[5,6,38,39]	Prevents mucosal clearance, antiphagocytic, sterically inhibits complement and immunoglobulin-binding to host receptors	4+
Pneumolysin[7,40]	Cytolytic, TLR4 ligand, induces ciliostasis, impairs respiratory burst, activates complement, cytokine, chemokine production	3+
Pneumococcal surface protein A[41]	Blocks C3b binding to factor B, binds to epithelial membranes	2+
Pneumococcal surface protein C; also known as choline binding protein A[42,43]	Binds factor H and blocks C3b fixation, binds to human polymeric immunoglobulin receptor during invasion	2+
Cell wall polysaccharide[5]	Activates complement, pro-inflammatory	1+
Pneumococcal surface antigen A[5,22,25]	Mediates metal ion uptake (zinc and manganese), protects against oxidant stress, binds to GlcNac-Gal	1–2+
Lipoteichoic acid[44]	Binds to PAFR, TLR2 ligand, proinflammatory	2+
Autolysin[5]	Releases peptidoglycan, teichoic acid, pneumolysin other intracellular contents	2+
Hyaluronate lyase[45]	Degrades hyaluronan in the extracellular matrix	1+
Enolase[5]	Binds to fibronectin in host tissues	1+
Sortase A[46]	Links surface proteins to cell wall	1+
Pili[47]	Pili on cell surface inhibits phagocytosis, promotes invasion	1+
Pneumococcal adhesion and virulence[48]	Binds to plasminogen within host tissues	1+
Pneumococcal iron acquisition A and iron uptake A[5]	ABC transporters that acquire iron for bacterial growth and virulence	1+
Bacteriocin[5]	Inhibits bacterial competition, might have other cytotoxic actions	2+
Neuraminidase[45]	Contributes to adherence, removes sialic acids on host glycopeptides and mucin to expose binding sites	1+
Biofilm and competence[33]	Biofilm organisms upregulate CSP; competence genes contribute to pneumonia and meningitis	2+
IgA protease[5]	Degrades human IgA1	1–2+
Phosphorylcholine[44]	Binds to PAFR on human epithelial cells	2+
Pneumococcal serine-rich repeat protein[25]	Surface adhesion, binds to platelet surface and promotes tissue invasion	2+
Pneumococcal choline binding protein A[49]	Low manganese-induced protein, unknown function but important in lungs and blood	1–2+

1+=one or few animal studies. 2+=some evidence from several animal studies. 3+=confirmed in several animal studies. 4+=confirmed in people. TLR=toll-like receptor. C3b=complement component 3b. GlcNac-Gal=N-acetylglucosamine-beta-(1-4)-galactose disaccharide. ABC=ATP-binding cassette. CSP=competence-stimulating peptide. PAFR=platelet-activating factor receptor.

Table 1: **Known virulence factors in *Streptococcus pneumoniae* infection**

include the antiphagocytic and adherence properties of capsular polysaccharides, adherence factors, invasion genes, iron and other heavy-metal transporters, oxidative stress protection, host-defence evasion, pneumolysin production, bacteriocin production, and quorum sensing and biofilm formation (figure 3).[5,38–49]

Virulence expression varies with tissue site and population density. Sessile pneumococci residing inside biofilms are more able to induce meningitis and pneumonia but have less capacity to disseminate in blood than do those unattached and not inside biofilms. By contrast, free-living, unattached (or planktonic) bacteria are more likely to induce bacteraemic infection, but are less successful in causing pneumonia.[33] Many virulence factors probably contribute to invasive pneumococcal disease in people, but unequivocal evidence exists only for pneumococcal capsules—the target of present vaccine formulations.

Pneumococcal capsular antigen is the most important virulence determinant for pneumococci in experimental and clinical studies.[38] The capsule is crucial during colonisation, invasion, and dissemination from the respiratory tract. It prevents mechanical clearance by mucous secretion[38] and helps with transit of the organism to the epithelial surface. Capsular polysaccharide is highly negatively charged and sterically inhibits the interaction between phagocytic CR3 receptors to iC3b, and between Fcγ receptors to the Fc component of IgG fixed to pneumococci.[38,39] Pneumococcal capsules also restrict autolysis and reduce exposure to several antibiotics.[3,5]

Pneumococcal exotoxin-pneumolysin is expressed by almost all invasive strains of *S pneumoniae*. This pore-forming cytotoxin is released during autolysis as a soluble monomer that oligomerises on host membranes. Pneumolysin is lytic to host cells if sufficient amounts of it are generated. The toxin has many other pathological effects, including its ability to inhibit ciliary action of epithelial cells, activate CD4+ T cells, impair respiratory burst of phagocytic cells, induce production of chemokines and cytokines, stimulate complement fixation, and activate inflammation.[7,40] Pneumolysin-negative mutants of *S pneumoniae* are much less likely to produce lethal pulmonary infections than are wild-type

pneumococci.[5,40] Figures 3 and 4 show the role of this toxin and other virulence factors during invasion.

Mechanisms of host recognition

Pattern-recognition receptors are key components of the innate immune system.[50,51] They recognise conserved motifs expressed by pathogens that are referred to as pathogen-associated molecular patterns. Several of these receptors contribute to initiation of an effective, innate immune response to the pneumococcus (figure 4). C-reactive protein, an acute-phase protein, functions as a pattern-recognition receptor for *S pneumoniae*. It binds phosphorylcholine in the pneumococcal cell wall and activates complement.[52] In animal models,[53] human C-reactive protein protects against lethal infection with *S pneumoniae* infection, and in man probably contributes to host defence during bacteraemic pneumonia.

S pneumoniae uses the host-derived receptor for platelet-activating factor to cross from lung tissue into blood. This receptor recognises the phosphorylcholine determinant of its natural human ligand—platelet-activating factor. Pneumococci express phosphorylcholine in their cell wall that binds to this receptor, initiating bacterial uptake.[54] In mice deficient in this receptor, pneumococcal growth is impaired, bloodstream invasion is reduced, and survival is improved.[21,55] The dendritic cell-specific intercellular adhesion molecule (ICAM-3-grabbing nonintegrin [DC-SIGN] homologue SIGN-related 1 [SIGNR1]) is a C-type lectin implicated in capture of capsular polysaccharides from *S pneumoniae* by marginal-zone macrophages.[56] Although SIGNR1 is not usually expressed by alveolar macrophages, SIGNR1-/- mice are highly susceptible to pneumococcal infection and do not clear *S pneumoniae* from their lungs or blood.[57] SIGNR1 probably restricts bacterial growth during pneumococcal pneumonia with presentation of pneumococcal antigens to B cells by SIGNR1 on marginal-zone splenic macrophages. Antiphosphorylcholine IgM antibodies resulting from this presentation assist in clearance of pneumococci from the lung.[57,58]

Macrophage receptor with collagenous structure (MARCO) is a class A scavenger receptor expressed on alveolar macrophages that is able to bind and internalise *S pneumoniae* in vitro. MARCO-/- mice have a greatly reduced resistance against pneumococcal pneumonia, with accelerated growth of pneumococci and increased mortality.[59] Toll-like receptors (TLRs) have a central role as pattern-recognition receptors in initiation of cellular innate immune responses because they can detect many microbial pathogens at either the cell surface or in lysosomes and endosomes.[50,51] TLR2 is generally thought to be the most important pattern-recognition receptor for gram-positive pathogens (ie, peptidoglycan, lipoteichoic acid, and bacterial lipopeptides), but its importance for pneumococci is not fully understood. *S pneumoniae* cell-wall components are recognised by TLR2,[60–64] but TLR4 is the receptor for the proinflammatory effects of pneumolysin.[65–67] Unexpectedly,

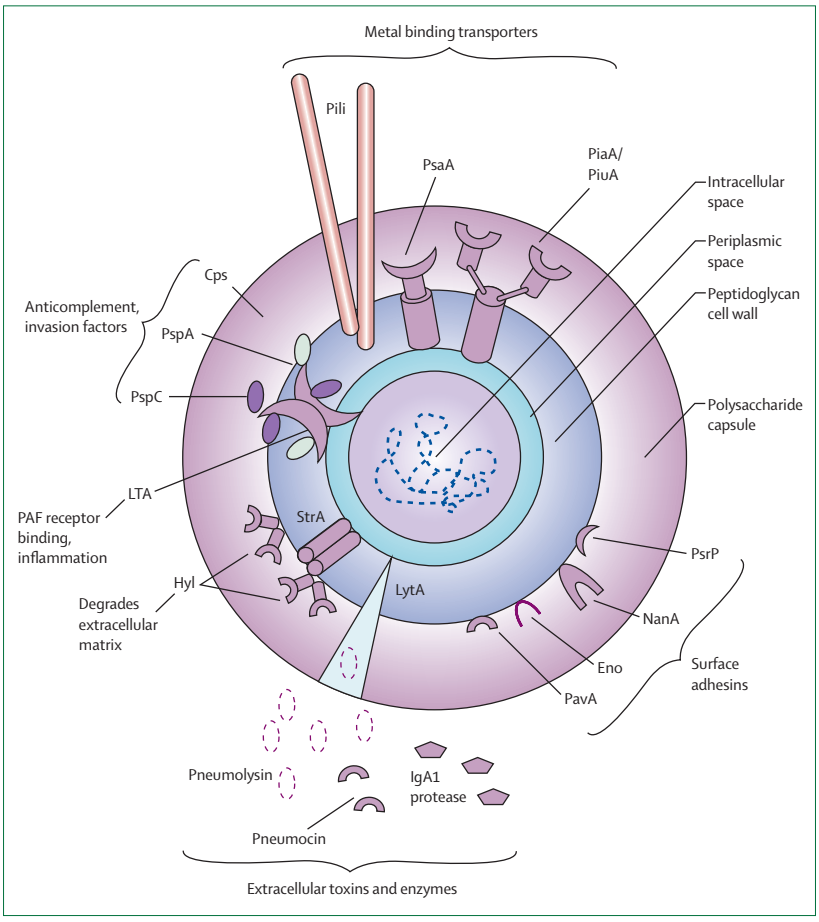

Figure 3: **Virulence factors of pneumococcus**
PsaA=pneumococcal surface antigen A. PiaA/PiuA=pneumococcal iron acquisition and uptake. PsrP=pneumococcal serine-rich repeat protein. NanA=neuraminidase. Eno=enolase. PavA=pneumococcal adhesion and virulence. LytA=autolysin. StrA=sortase A. Hyl=hyluronate lyase. LTA=lipoteichoic acid. PspC=pneumococcal surface protein C. PspA=pneumococcal surface protein A. Cps=polysaccharide capsule. PAF=platelet-activating factor.

when many different types of TLR-deficient mice were studied to investigate which of these receptors was most essential to detect and defend against pneumococci, only TLR9-/- mice were highly susceptible to lethal infection. TLR9 detects bacterial DNA and seems to be essential for effective phagocytosis and killing of pneumococci by lung macrophages.[68]

Intracellular signalling elements of the TLR system are essential for defence against pneumococcal pneumonia in experimental systems. Mice deficient in the common TLR-adaptor protein myeloid-differentiation primary-response protein 88 (MyD88) are very susceptible to pneumococcal pneumonia, probably in part as a result of impaired innate immune activation by interleukin 1 and interleukin 18.[69–71] The clinical relevance of these findings accords with the discovery of children with a genetic deficiency for MyD88 or interleukin 1 receptor-associated kinase 4 (IRAK4), a kinase acting directly downstream from MyD88, who are especially susceptible to invasive pneumococcal disease.[72,73] Additionally, a single nucleotide

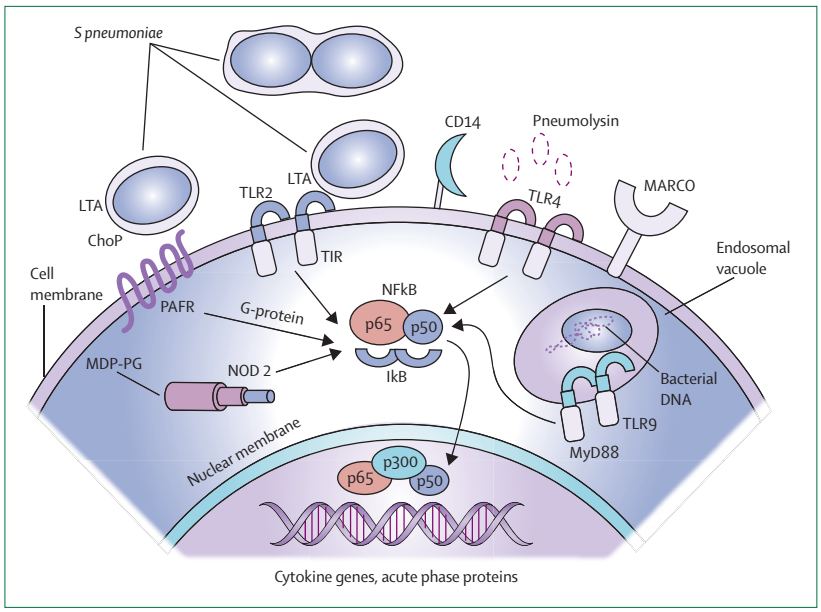

Figure 4: **Pattern-recognition signalling receptors and pathways in pneumococcal infection**
S pneumoniae is recognised as a pathogen in the lung by several toll-like receptors (TLRs), including TLR2 (with pneumococcal lipoteichoic acid [LTA] as its major ligand), TLR4 (recognises pneumolysin), and TLR9 (within endosomes; interacts with bacterial DNA). Macrophage receptor with collagenous structure (MARCO) expressed by alveolar macrophages contributes to innate immune response in lungs. PAFR is shown as a pattern-recognition receptor because it recognises pneumococcal phosphorylcholine and LTA, thereby contributing to tissue invasion. (Soluble) cluster-determining (CD) 14 probably further helps *S pneumoniae* invade from the airways into blood. Within cytoplasm, the muramyl dipeptide component of pneumococcal peptidoglycan (MDP-PG) is recognised by nucleotide-binding oligomerisation domain (NOD-2) and can activate host defence and inflammation. ChoP=phosphorylcholine. PAFR=platelet-activating factor receptor. TIR=toll-interleukin-1 receptor domain. MyD88=myeloid differentiation primary response protein-88. NFκB=nuclear factor κB. IκB=inhibitor κB.

polymorphism in the TLR-adaptor-protein Mal affects host defence against *S pneumoniae* in people.[74] CD14 is another crucially important pattern-recognition receptor.[22] CD14-/- mice are strongly protected against dissemination of *S pneumoniae* from the respiratory tract,[22] suggesting that pneumococci specifically use CD14 in the bronchoalveolar compartment to spread.

Pneumococci are slowly killed inside phagolysosomes, especially if poorly opsonised,[75] and this process might provide an opportunity for viable organisms or their bacterial products to escape from early phagosomes and invade the cytosol. Within the intracellular space, pneumococci can be recognised by various cytoplasmic pattern-recognition receptors. Nucleotide-binding oligomerisation domain protein (NOD2) recognises muramyl dipeptide, a common fragment of bacterial peptidoglycan, in the cytosol.[50,76] Additionally, bacterial infection leads to activation of caspase-1 by a protein complex called inflammasome.[77] Inflammasome regulates activity of caspase-1, an enzyme responsible for secretion of three major host-defence cytokines—interleukin 1β, interleukin 18, and interleukin 33. However, caspase-1-/- mice seem to tolerate pneumococcal pneumonia.[68] Thus, the clinical implications of NOD2 and inflammasomes in pneumococcal infections in people remain unclear.

Immunology

Respiratory epithelial cells not only provide the mucociliary carpet to continually remove potential pathogens from the lower airways, but also actively respond to the presence of pathogens. Respiratory epithelium releases various mediators such as cytokines, chemokines, and antimicrobial peptides (eg, lysozyme, defensins, and cathelicidins), contributing to innate immunity against pneumococci.[78] Transgenic mice that overexpress nuclear factor inhibitor κB-α (IκB-α) and block nuclear factor κB (NFκB) nuclear translocation within epithelial cells have a reduced ability to clear pneumococci from the airways.[79] Alveolar lining cells produce a pneumococcal-binding protein known as surfactant protein-D (SP-D). Mice deficient in this surfactant protein have a decreased capacity for clearance of pneumococci and are prone to disseminated infection.[80]

Alveolar macrophages represent the first phagocytic defence in the lungs and can phagocytise and kill low numbers of pneumococci.[75,81] When large numbers of pneumococci are introduced into the lower airways, neutrophils are recruited and they become the main phagocytic cells in the acutely inflamed lung. Alveolar macrophages are then relegated to clearing apoptotic neutrophils.[82] They also undergo apoptosis during pneumococcal pneumonia—macrophage apoptosis helps with killing of phagocytised *S pneumoniae*[83] and keeps pneumococcal invasion into the bloodstream to a minimum.[66,81]

Neutrophils predominate within cellular infiltrates in pneumococcal pneumonia. The conventional notion of neutrophil migration with selectin-mediated rolling and β2-integrin-mediated tight adhesion to the endothelium does not apply to pneumococcal pneumonia.[84] β2-integrin-deficient mice show normal neutrophil trafficking into lung tissue after infection with *S pneumoniae*.[85] Another host-derived, soluble adhesion molecule known as galectin-3 has been implicated as a major neutrophil recruitment signal in pneumococcal pneumonia.[86] Galectin-3-/- mice with *S pneumoniae* have accelerated lung infection, with early disseminated disease.[87] Additionally, galectin-3 augments neutrophil phagocytosis and exerts bacteriostatic effects on *S pneumoniae*.

α chemokines also promote an influx of neutrophils into lung tissue during pneumococcal pneumonia,[88] whereas reactive oxygen species derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase restrict neutrophil reflux.[89] Moreover, pneumococci produce chemotactic factors such as N-formyl-methionyl-leucyl-phenylalanine and pneumolysin that help with neutrophil recruitment.[88–90] The net effect to a host of neutrophil influx in pneumococcal pneumonia can be good or bad, dependent on the virulence of the pathogen. Antineutrophil antibody treatment resulted in widespread infection and increased mortality with serotype 3 pneumococci.[91] Depletion of neutrophils

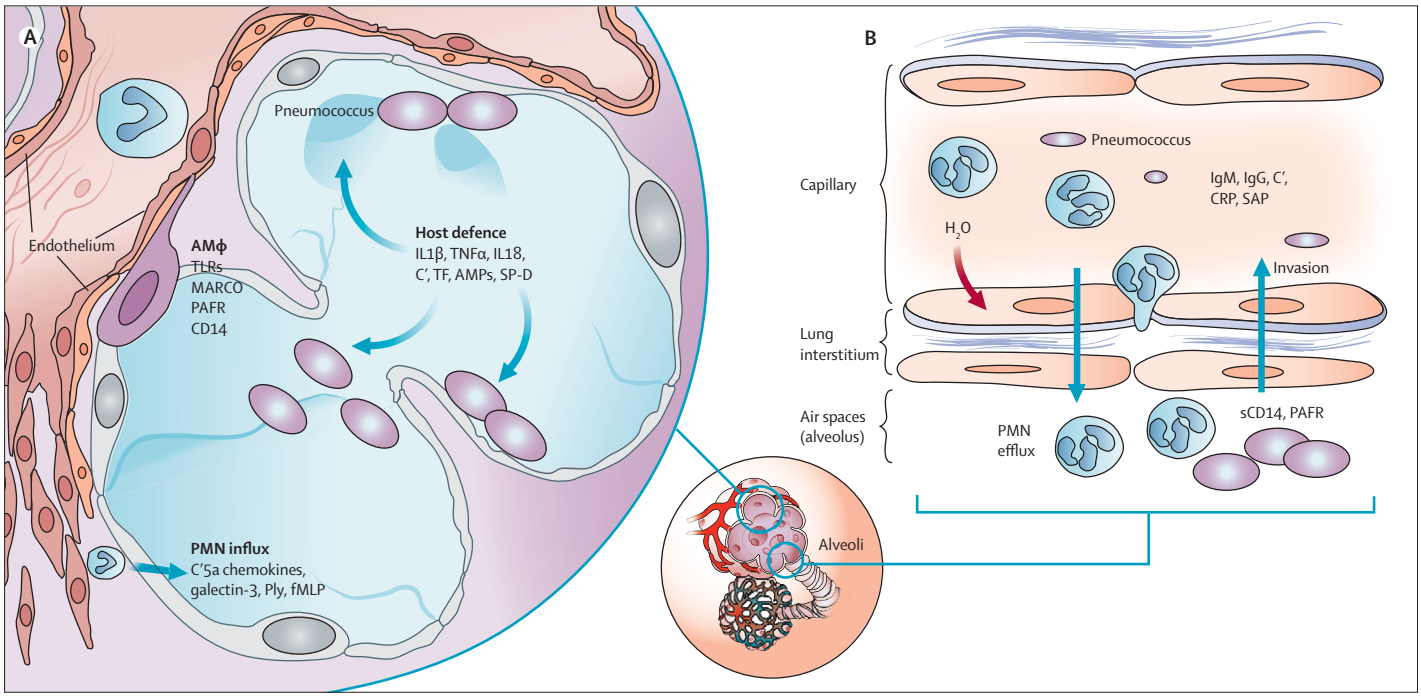

Figure 5: **Major pathological events in invasive pneumococcal pneumonia**

(A) Pneumococci that enter lower airways are recognised by pattern-recognition receptors, including toll-like receptors (TLRs; on epithelial cells and alveolar macrophages) and macrophage receptor with collagenous structure (MARCO; on alveolar macrophages). At low infectious doses, epithelial cells and alveolar macrophages can clear *S pneumoniae* without help from recruited neutrophils, in part by release of protective inflammatory mediators such as interleukin 1 (IL 1), tumour necrosis factor α (TNF α), interleukin 18 (IL 18), complement products (C'), surfactant protein-D (SP-D), and antimicrobial peptides (AMPs). These mediators continue to have a role after infection with a high infectious dose, whereby polymorphonuclear cells (PMN) are recruited by chemoattraction through various mediators including C'5a, and galectin-3, and pneumococcal products such as pneumolysin (Ply) and formyl-methionine-leucine-phenylalanine (fMLP). (B) If alveolar defence mechanisms are overwhelmed by multiplication of pneumococci, invasion of *S pneumoniae* into the bloodstream takes place, helped by platelet-activating factor receptor (PAFR) and (soluble) CD14 (sCD14). In the bloodstream, several host proteins contribute to host defence including natural IgM antibodies, C', C reactive protein (CRP), and serum amyloid (SAP). TF=tissue factor. AMφ=alveolar macrophage.

worsened outcomes in those with a high infectious dose of serotype 4 *S pneumoniae*, but not in those with a low inoculum.[92] By contrast, neutrophil depletion in mice with a serotype 8 strain improved survival and resulted in reduced bacteraemia.[93] Pneumococcal pneumonia is uncommon in adults with isolated neutropenia and without other concomitant immune defects.

The greatly increased frequency of pneumococcal pneumonia in patients with AIDS attests to the protective role of CD4+ T cells in resistance to pneumococci. *S pneumoniae* elicits an early accumulation of T cells in lung infection[94,95]—a response that is dependent on pneumolysin. MHCII-/- mice have a pronounced deficiency in CD4+ T cells and are highly susceptible to pneumococcal pneumonia.[95] γδ T cells make up only 2% of circulating T cells but are present in increased concentrations in respiratory tissues. Pneumococcal pneumonia is associated with an increase in γδ T-cell subsets in lung tissues.[96,97] Vγ4 T cell-/- mice are very susceptible to pneumococci and show reduced trafficking of neutrophils into lung tissue.[96] γδ T cells also contribute to the resolution phase of *S pneumoniae* infection.[97] Natural killer T cells have a crucially important role in defence against pneumococcal pneumonia.[98] Treatment of mice with α-galactosylceramide, which specifically activates Vα14+ natural killer T cells, improves clearance of pneumococci.[99]

Clearance of pneumococci from the circulation strongly depends on opsonisation by complement components and phagocytosis by myeloid cells.[1,3] Disruption of the common C3 pathway profoundly impairs resistance to pneumococci.[85] Of the three complement cascades (classic, alternative, and mannose-binding lectin pathways), the classic pathway has been identified as most important in host defence against pneumococci.[100] Natural IgM antibodies contribute to innate immune responses through activation of the classical complement pathway.[100] Antibody-independent activation of the classic pathway also arises through SIGNR1,[101] C-reactive protein,[52] and serum amyloid P.[102]

Recognition of pneumococci by immune cells within the respiratory tract generates an array of proinflammatory and anti-inflammatory cytokines. Some of these cytokines play a pivotal part in innate defence against pneumococci. Tumour necrosis factor α (TNFα) is of particular importance because its inhibition or elimination greatly helps with growth and dissemination of pneumococci[103]—an effect mainly mediated by the type I TNF receptor.[104] Interleukin 1 seems to have a similar yet less important protective role.[70] Inhibition of both TNF and interleukin 1 renders animals very susceptible to pneumococcal pneumonia and other types of bacterial infections.[70,105] Other proinflammatory cytokines that are important for

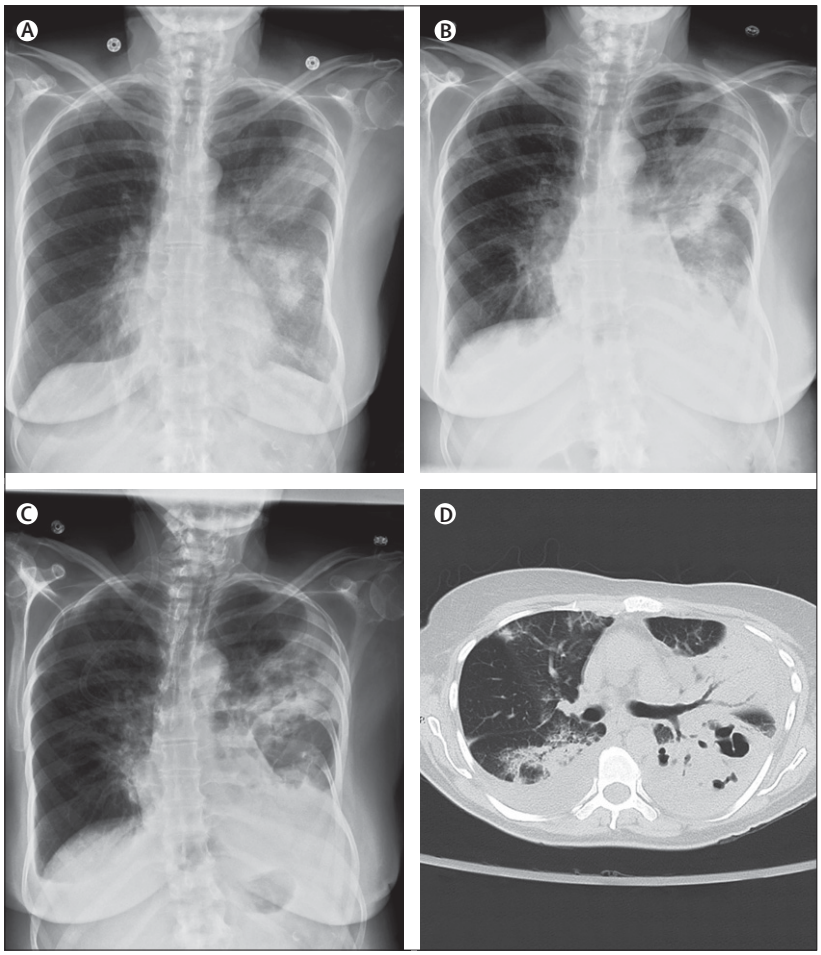

Figure 6: **Typical radiographic findings in pneumococcal pneumonia**
This previously healthy woman aged 73 years presented to hospital with bacteraemic multilobar left-sided pneumococcal pneumonia (A). 5 days later, the infiltrate worsened (B) despite appropriate therapy (ceftriaxone and clarithromycin) for this penicillin-susceptible strain of *S pneumoniae*. By day 10, chest radiograph (C) shows a parapneumonic effusion, extensive consolidation, and areas of focal cavitation. A chest CT image on day 12 (D) confirms cavitation and consolidation with air bronchograms. The patient slowly recovered with antimicrobial therapy alone and had a chest radiogram after 2 months that was clear apart from minor parenchymal scarring in the left lower lobe.

defence against pneumococci are interleukin 6 and interleukin 18,[71,106] whereas the anti-inflammatory cytokine interleukin 10 impairs defence mechanisms during both primary and postinfluenza pneumococcal pneumonia.[98]

Surprisingly, interferon γ inhibits antibacterial defence in models of either primary or postinfluenza pneumococcal pneumonia,[91,107] possibly because this cytokine decreases the capacity of alveolar macrophages to kill *S pneumoniae*.[91] Other investigators[108,109] have reported widely disparate results with blockers of interferon γ. Interleukin 12, a prominent inducer of interferon γ, has been reported to either enhance[109] or have no effect[77] on host defence against pneumococcal pneumonia. Together, these studies show that cytokine-mediated enhancement of lung inflammation improves outcomes for animals with *S pneumoniae* pneumonia, although interferon γ might worsen outcomes.

S pneumoniae pneumonia results in activation of nuclear factor κB within the lungs.[110] Inhibition of nuclear factor κB activation increases lethality and enhances bacterial growth in animal models of infection with *S pneumoniae*.[79,111] A role for adequate activation of this factor in defence against pneumococci in people is supported by the association between common polymorphisms in the inhibitor κB genes *NFKBIA* and *NFKBIE*, and increased susceptibility to invasive pneumococcal disease.[112] Extensive cross-talk exists between the coagulation system and the innate immune system in response to microbial infection.[113] These systems simultaneously activate and collaborate to contain and eradicate localised infection.[114] Pneumococcal pneumonia is associated with both intrapulmonary and systemic activation of the coagulation system—a response initiated by tissue factor.[115,116] Anticoagulant treatment with recombinant tissue-factor pathway inhibitor, activated protein C, or antithrombin greatly restricts lung coagulopathy in pneumococcal pneumonia in animals.[117] Infusion of recombinant human-activated protein C improves 28 day mortality in patients with severe sepsis and reduces the high likelihood of dying.[116] This beneficial effect was most evident in a subgroup of patients who had pneumococcal pneumonia.[116] Figure 5 shows the host responses within the lung induced by *S pneumoniae*.

Clinical features

Pneumococcal pneumonia usually presents as typical, acute community-acquired pneumonia. It generally begins with a mild upper-airway irritation attributable to a respiratory viral infection. When pneumococci are deposited into the lower airways they are usually expelled by mucociliary clearance, cough, antimicrobial peptides, and local innate immune defences. Should these systems fail to eliminate the pathogen, systemic inflammation ensues with characteristic signs and symptoms of bacterial pneumonia.[1,3] Onset of severe illness is abrupt, and develops with a shaking chill, fever, malaise, cough, and dyspnoea. The cough becomes productive with purulent sputum, sometimes with brownish or blood-tinged sputum with respirophasic chest pain and progressive dyspnoea. Left untreated, this toxic illness can progress to acute respiratory failure, septic shock, multiorgan failure, and death within several days from onset.

Figure 6 shows typical radiographic findings in an elderly patient with bacteraemic pneumococcal pneumonia. Resolution of radiographic findings for this illness is slow, with abnormal findings in half of patients up to 6 weeks after symptom onset.[118] Need for and clinical usefulness of routine follow-up radiographs after uncomplicated pneumococcal pneumonia is questionable and no longer recommended.[119–121] Recurrent or non-resolving pneumonia in the same anatomical location is suggestive of a possible endobrochial lesion.

Recurrent pneumococcal pneumonia in different bronchopulmonary segments should prompt a search for an underlying immunocompromised state. HIV infection, congenital or acquired B-cell disorders, and ciliary dyskinesia are common concerns in patients younger than 18 years, and multiple myeloma and other ymphoreticular disorders are suspected in patients older than 65 years.[118]

Clinical presentation of pneumococcal pneumonia might not be typical in some vulnerable patients, and diagnosis can be difficult. Invasive pneumococcal disease can be subtle in its early phases in neonates, elderly people, severely immunocompromised patients, asplenic hosts, and in various concomitant co-morbid illnesses.[118,122] Patients can present with extrapulmonary symptoms (meningitis, overwhelming sepsis, pericarditis, peritonitis, mastoiditis, and endocarditis) before showing evidence of bacterial pneumonia.

Diagnosis

Diagnostic methods for pneumococcal pneumonia have not changed appreciably since Pasteur and Sternberg first isolated *S pneumoniae* in 1881, and Christian Gram used his famous stain to reveal pneumococci under the microscope in 1886 (figure 7). Pneumococci grow readily on blood agar plates in a CO_2 incubator at 37°C. *S pneumoniae* colonies are α haemolytic and often umbilicate because of autolysis. Cultures from sputum, blood, and other tissue sites should be obtained before empirical antibiotic therapy is started.[118]

Two innovations for rapid diagnosis of pneumococcal disease are now available and can be quite useful. The first is urinary antigen detection of the C polysaccharide from the pneumococcal cell wall by an immunochromatography assay. This test compares favourably with culture and gram stain for detection of invasive pneumococcal disease.[123] The assay is most sensitive in severe pneumococcal disease and bacteraemia, and can be falsely negative in early pneumococcal infection. This assay is especially useful in patients from whom clinicians have difficulty getting an adequate sputum sample, and in those who have already begun antibiotic treatment before cultures were obtained. The urinary antigen remains positive for weeks after onset of severe pneumococcal pneumonia. Major drawbacks of this assay are an inability to acquire antimicrobial susceptibility data from this test, and loss of sensitivity with mild infections. The second is the many non-culture assays based on nucleic acids for pneumococcal pneumonia that are now available or in development. They are rapid and highly specific, with *S pneumoniae*-specific DNA sequences as targets for detection assays.[124,125] To distinguish pneumococcal colonisation from infection remains a challenge. Perhaps these rapid diagnostic techniques will be useful for making management decisions about patients with pneumococcal infections in the future.

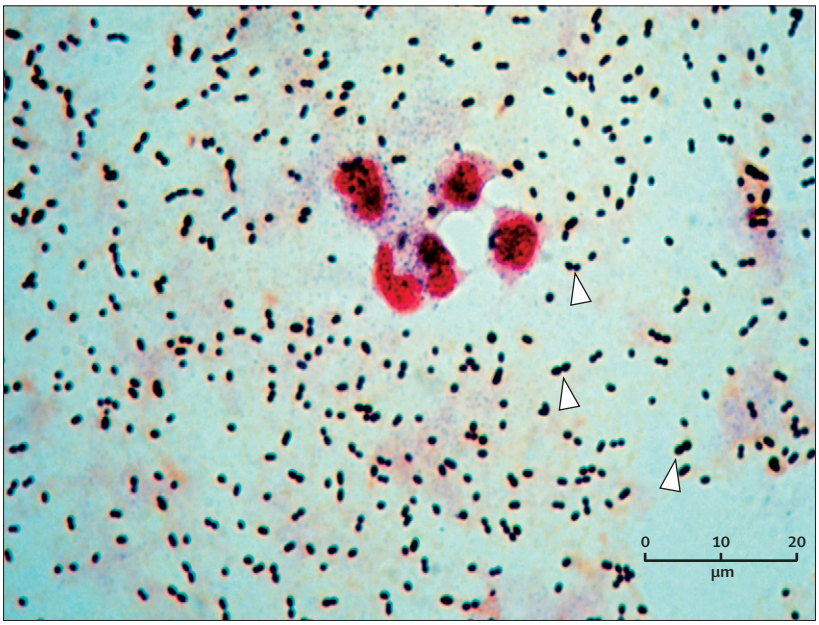

Figure 7: **Classic gram stain findings in acute pneumococcal pneumonia**
Note numerous lancet-shaped gram-positive diplococci and neutrophilic cellular infiltrate. Pneumococci as diplococci (arrows). (High power magnification under oil immersion ×1000.)

Treatment

The initial, uniform activity of penicillin against *S pneumoniae* resulted in this antibiotic being treatment of choice for community-acquired pneumonia since the late 1940s.[1] Penicillin-resistant strains of *S pneumoniae* were first noted in the mid 1970s and resistant clones have now spread worldwide.[1,118,126] Penicillin resistance is related to structurally modified penicillin-binding proteins of *S pneumoniae*. These modified binding proteins allow peptidoglycan synthesis despite the presence of penicillin. This resistance mechanism can often be overcome with high doses of penicillin. Present clinical laboratory standards that define the minimum inhibitory concentration for susceptibility, intermediate resistance, and high-level resistance to penicillin vary somewhat by region (table 2).[118,120,121]

Non-meningeal *S pneumoniae* strains expressing intermediate and even high-level resistance to penicillin can still be treated with high-dose, β-lactam antibiotics (penicillins, or second or third generation cephalosporins).[121,126,127] Meningitis attributable to *S pneumoniae* with even intermediate resistance to pneumococci necessitates use of other agents to assure a successful outcome.[118] Table 2 provides the present recommended treatment regimens and comparisons between the British,[120] European,[121] and American[118] guidelines for community-acquired pneumonia and specific types of *S pneumoniae* infections.

Antimicrobial resistance of pneumococci to macrolides,[128] fluoroquinolones,[129] vancomycin,[126] trimethoprim,[118] and various other antimicrobial agents is increasingly recognised worldwide.[126,127] When feasible,

	Primary therapy	Alternative therapies
Community-acquired pneumonia with PCN-S or PCN-I *S pneumoniae*	BTS: 500 mg ampicillin every 6 h or benzylpenicillin 1–2 g every 6 h; ATS; penicillin G 6–10 million units per day; ERS: penicillin, or second or third generation cephalosporin, with or without macrolide	Second or third generation cephalosporin; if β-lactam allergy give clarithromycin, azithromycin, or fluoroquinolone
Community-acquired pneumonia with PCN-R *S pneumoniae*	BTS: high-dose penicillin or second or third generation cephalosporin or alternative therapy; ATS: high-dose second or third generation cephalosporin or fluoroquinolone; ERS: fluoroquinolone, vancomycin, or linezolid	Vancomycin, fluoroquinolones, linezolid, carbapenems if susceptible
Pneumococcal bacteraemia with sepsis or meningitis, PCN-S/I *S pneumoniae*	High-dose penicillin or ampicillin, with or without macrolide or fluoroquinolone; add dexamethasone for pneumococcal meningitis	High-dose second or third generation cephalosporin; if β-lactam allergy give vancomycin, fluoroquinolones
Pneumococcal bacteraemia with sepsis or meningitis, PCN-R *S pneumoniae*	High-dose third generation cephalosporin with or without macrolide or fluoroquinolone; if meningitis add vancomycin with or without rifampin+dexamethasone	If β-lactam allergy give vancomycin with or without rifampin; consider TMP-SMX or chloramphenicol in meningitis if susceptible
Suspected pneumococcal infection or overwhelming post-splenectomy infection	Immediate treatment with oral amoxicillin and clavulanate, seek immediate attention	If β-lactam allergy, immediate treatment with oral clarithromycin, seek medical attention
Invasive pneumococcal disease and immunoglobulin deficiency	Standard antimicrobial therapy	Consider adding intravenous immunoglobulin 1–2g/kg with antimicrobial therapy

Recommendations for patients with community-acquired pneumococcal pneumonia and others invasive diseases. Recommendations based on guidelines from the British Thoracic Society (BTS),[120] Infectious Diseases Society of America/American Thoracic Society (ATS),[118] European Respiratory Society (ERS).[121] PCN=penicillin. S=susceptible. I=intermediate. R=resistant. TMP-SMX=trimethoprim-sulfamethoxazole. Fluoroquinolone=respiratory fluoroquinolones (moxifloxacin or levofloxacin), not ciprofloxacin because of high resistance rates. Minimum inhibitory concentration (MIC) breakpoints for lung isolates: BTS >0·1 mg/L (S), 0·1–1·0 mg/L (I), and >1–4 mg/L (R); ERS <0·5 mg/L (S), 0·5–2·0 mg/L (I), >2·0 (R); ATS ≤2·0 μg/mL (S), 2–4 μg/ml (I), ≥8 μg/ml (R). For meningeal isolates: ATS <0·06 μg/mL (S), 0·12–1·0 μg/ml (I), and ≥2 μg/mL (R). ERS guidelines regard cerebrospinal fluid isolates with MIC <0·06 mg/L=susceptible, >0·06 mg/L= resistant.

Table 2: **Treatment recommendations for parenteral therapy for patients admitted to hospital**

S pneumoniae strains isolated from patients who are sufficiently ill to need hospital admission should undergo sensitivity testing because susceptibilities to standard agents are no longer assured.[126] This testing is especially important in assessment of blood or cerebrospinal fluid isolates, and in geographical areas where resistance to standard antibiotics is a known problem.

Much controversy exists about the advisability of use of combination therapy with a β-lactam and either a macrolide or respiratory fluoroquinolone for bacteraemic pneumococcal pneumonia. Results of several retrospective and small prospective studies[130–132] show a possible survival advantage with combination therapy, even when the pneumococcal isolate is susceptible to β-lactams. Some form of in-vivo synergy might exist, or combination therapy might treat some unrecognised co-pathogens not covered by β-lactams (eg, *Mycoplasma pneumoniae*, *Chlamydophila* spp, or other untypical pathogens). Macrolides and perhaps even fluoroquinolones might limit excessive host-derived inflammatory reactions to severe pneumococcal pneumonia.[130–132] In a laboratory study,[133] researchers reported improved outcomes and reduced lung inflammation when inhibitors of bacterial protein synthesis (clindamycin or azithromycin) were added to ampicillin for postinfluenza pneumococcal pneumonia. These protein inhibitors might reduce synthesis of microbial mediators that contribute to lung inflammation. Convincing clinical evidence from large numbers of people to show that combination therapy is better than monotherapy is highly desirable. Individual decisions about dual therapy for severe invasive pneumococcal disease presently rest on available but insufficient clinical information.

Early intervention with effective antimicrobial agents provides a substantial survival advantage in severe pneumococcal pneumonia. Antibiotics should be started as soon as possible (<4–6 h) after a patient enters a health-care facility for the best outcome.[118,120,121] Optimum supportive care with supplemental oxygen, ventilatory support, volume resuscitation, vasopressors, supportive nutrition, and other measures can be lifesaving. Although controversy remains about the risk–benefit ratio for recombinant-activated protein C in severe sepsis, this therapy is still an option in severe pneumococcal pneumonia.[116]

Vaccine strategies

Prevalence and intrinsic virulence of pneumococci, and progressive resistance to antimicrobial agents has rekindled an interest in improved vaccines against *S pneumoniae*. Two vaccine formulations are available to prevent pneumococcal infection. The polysaccharide vaccine consists of the 23 most common capsular serotypes that cause invasive pneumococcal disease in the developed world.[1,3,5] This vaccine induces T-cell-independent B-cell responses and is in widespread use.[118] Its effectiveness is hampered by poor vaccine responses in elderly people, immuno-

compromised patients, and in infants younger than age 2 years—precisely those at greatest risk for severe pneumococcal disease.

The covalently linked polysaccharide-protein conjugate pneumococcal vaccine has been in use for almost 10 years and was very successful[16,17] (figure 1). The seven-valent conjugate vaccine (Prevenar; Wyeth) is approved for use mainly in children younger than 2 years, and for children younger than 5 years with high-risk conditions. This vaccine targets the pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. These seven serotypes are responsible for 80% of pneumococcal infections in children living in developed countries. The vaccine is highly immunogenic in young children because it is T cell-dependent and overcomes the intrinsic loss of immunogenicity to polysaccharide vaccines within the first 2 years of life.[16] This vaccine has reduced incidence of invasive pneumococcal disease in children in the USA younger than 1 year by 82%.[17] One of the most remarkable attributes of the pneumococcal conjugate vaccine has been the degree of herd immunity it generates. Frequency of invasive pneumococcal disease infection in non-vaccinated siblings and even adult contacts has declined substantially.[16,134,135] Young children are the primary reservoir for pneumococcal colonisation in people, and thus elimination of the carrier state in children reduces risk of transmission to the rest of the population.

Despite the effectiveness of the conjugate vaccine, disturbing patterns are developing in the epidemiology of pneumococcal disease that threaten the long-term benefits of the vaccine. Invasive disease attributable to non-vaccine serotypes of *S pneumoniae* has greatly increased.[18] Vaccine-induced anticapsular antibodies restrict access of vaccine-associated serotypes to the human upper airways. This niche is now available to non-vaccine pneumococcal serotypes or other oropharyngeal pathogens such as *Staphylococcus aureus*.[16–18] Vaccine replacement by non-virulent serotypes would be acceptable, but virulent, non-vaccine serotypes can also replace vaccine serotypes of pneumococci.[3,136,137] Capsular switching probably arises through transformation of capsular genes from one serotype to another.[18,138–140] Such events could arm an intrinsically virulent strain of *S pneumoniae* previously encapsulated with a vaccine-related serotype with a new antigenic polysaccharide coat to avoid vaccine-induced immune recognition.

Analysis of patterns of serotype replacement over time after widespread institution of the seven-valent conjugate vaccine is needed. An expanded conjugate vaccine including serotypes not covered in the present vaccine might be useful. Serotype 19A *S pneumoniae* is a specific concern.[136] This serotype has expanded greatly since introduction of the conjugate pneumococcal vaccine,[138,139] and has much virulence potential as a pulmonary and extrapulmonary pathogen. Moreover, it has the propensity to acquire multiple drug-resistance genes, complicating antimicrobial therapy against this organism.[139,140] Other vaccine approaches include development of vaccines against many, highly conserved, immunogenic protein antigens—eg, adhesins, pneumolysin, invasion proteins, and transport proteins.[5]

Conclusions

The pneumococcus fascinates immunologists, yet frustrates clinicians and public-health officials attempting to control it. Although the human respiratory tract has many local and systemic immune defences, a range of pneumococcal virulence factors work together to cause invasive disease. The capacity of *S pneumoniae* to resist antimicrobial agents and escape immune defences shows that control of this pathogen will not be easy to achieve. Thus a multifaceted approach with new generation vaccines, novel antimicrobial therapies, and improved adjuvant treatments will be needed.

Contributors

Both authors contributed equally to this Seminar.

Conflicts of interest

We declare that we have no conflicts of interest.

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