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Pathophysiological role of respiratory dysbiosis in hospital-acquired pneumonia

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Department of Anesthesiology and Critical Care, CHU Nantes, Nantes, France

(Prof A Roquilly MD,

Prof K Asehnoune MD);

Department of Microbiology and Immunology, Faculty of

Medicine, University of

Nantes, Nantes, France

(Prof A Roquilly,

Prof K Asehnoune); *Servei de*

Pneumologia, Hospital Clinic,

Universitat de Barcelona

Institut d'investigació

Biomèdica August Pi i Sunyer,

Centro de Investigación

Biomédica en Red.

Enfermedades Respiratorias,

Barcelona, Spain

(Prof A Torres MD); Department

of Microbiology and

Immunology, Doherty

Institute of Infection and

Immunity and Department of

Biochemistry and Molecular

Biology, Bio21 Molecular

Science and Biotechnology

Institute, The University of

Melbourne, Parkville, VIC,

Australia

(Prof J A Villadangos PhD);

Department of Internal

Medicine, Radboud University

Medical Center, Nijmegen,

Netherlands

(Prof M G Netea MD); Division

of Pulmonary and Critical Care

Medicine, Department of

Internal Medicine, University

of Michigan Medical School,

Ann Arbor, MI, USA

(Prof R Dickson MD); Michigan

Center for Integrative Research

in Critical Care; Ann Arbor, MI,

USA (Prof R Dickson); and

Institute of Experimental

Immunology, University of

Zurich, Zurich, Switzerland

(Prof B Becher PhD)

Correspondence to:

Prof Karim Asehnoune,

Department of Anesthesia and

Critical Care, CHU Nantes,

44093 Nantes, France

[karim.asehnoune@chu-](mailto:karim.asehnoune@chu-nantes.fr)

[nantes.fr](mailto:karim.asehnoune@chu-nantes.fr)

Hospital-acquired pneumonia is a major cause of morbidity and mortality. The incidence of hospital-acquired pneumonia remains high globally and treatment can often be ineffective. Here, we review the available data and unanswered questions surrounding hospital-acquired pneumonia, discuss alterations of the respiratory microbiome and of the mucosal immunity in patients admitted to hospital, and explore potential approaches to stratify patients for tailored treatments. The lungs have been considered a sterile organ for decades because microbiological culture techniques had shown negative results. Culture-independent techniques have shown that healthy lungs harbour a diverse and dynamic ecosystem of bacteria, changing our comprehension of respiratory physiopathology. Understanding dysbiosis of the respiratory microbiome and altered mucosal immunity in patients with critical illness holds great promise to develop targeted host-directed immunotherapy to reduce ineffective treatment, to improve patient outcomes, and to tackle the global threat of resistant bacteria that cause these infections.

Introduction

Patients admitted to hospital for severe medical or surgical conditions, such as severe brain injury or sepsis, are susceptible to secondary hospital-acquired infections, which are a major health concern worldwide. In 2017, the European Center for Disease Prevention and Control estimated that more than 2.5 million cases of hospital-acquired infection occur annually in Europe, accounting for a substantial proportion of disability and premature

death.¹ Hospital-acquired pneumonia is the most common cause of nosocomial infection, and 500 000 episodes occur every year in Europe. The medical consequences of hospital-acquired pneumonia are severe, and include prolonged time spent admitted to hospital, long-term asthenia and depression, and increased mortality.² The economic burden of hospital-acquired pneumonia is high because the average cost for each episode is 40 000 euros in Europe, and annual medical expenses reach USD\$8 billion per year in the USA.³

Currently, the prevention and treatment of hospital-acquired pneumonia is mostly aimed at reducing the bacterial load, in particular by using antimicrobial therapy.^{4,5} No adjunctive non-antibiotic therapy is recommended. With the emergence of multi-resistant bacterial pathogens in hospital-acquired pneumonia, which are difficult to treat, the prevention and treatment of the infection will become even less efficient in the next decade.⁶ A reappraisal of the prevention and treatment of hospital-acquired pneumonia is urgently required.

Developments in understanding the physiology of healthy lungs, notably host–pathogen and microbiome interactions, have paved the way toward a better understanding of hospital-acquired pneumonia physiopathology. This reappraisal may lead to the development of targeted strategies with the potential to enhance patient outcomes and to diminish the risk of bacterial resistance.

Hospital-acquired pneumonia is distinct from community-acquired pneumonia; microorganisms that are involved in hospital-acquired pneumonia are different to the pathogens that cause community-acquired pneumonia (which are frequently virulent and transmitted by inhaled aerosols, legionella, viruses, atypicals). Moreover, respiratory physiology is severely altered in patients admitted to hospital. Severe medical conditions and interventions (such as mechanical ventilation) can in only a few hours trigger cilia impairment that disrupts the movement of mucus towards the trachea,⁷ and the positive pressure applied by

Key messages

- The prevention and treatment of hospital-acquired pneumonia, which has historically been based on bacterial contamination of sterile airways is efficient, but can be improved
- Lungs harbour a diverse and dynamic ecosystem of bacteria that continuously interact with mucosal immunity
- The biomass of the lung microbiome increases over time in patients who are mechanically ventilated, but its diversity decreases before the clinical development of hospital-acquired pneumonia
- The microbiome can be therapeutically manipulated, rather than eliminated by antibiotics, to prevent or resolve hospital-acquired pneumonia
- Studies in patients admitted to hospital who were considered immunocompetent have revealed substantial alterations in the function of several immune cells, which are associated with critical illness-related immunosuppression
- The development of therapies that aim to restore immune function, without directly targeting pathogens, have the potential to reduce the medical burden of hospital-acquired pneumonia
- Prevention and treatment of hospital-acquired pneumonia should aim to normalise the diversity of the microbiome and to restore mucosal immunity, rather than to sterilise airways

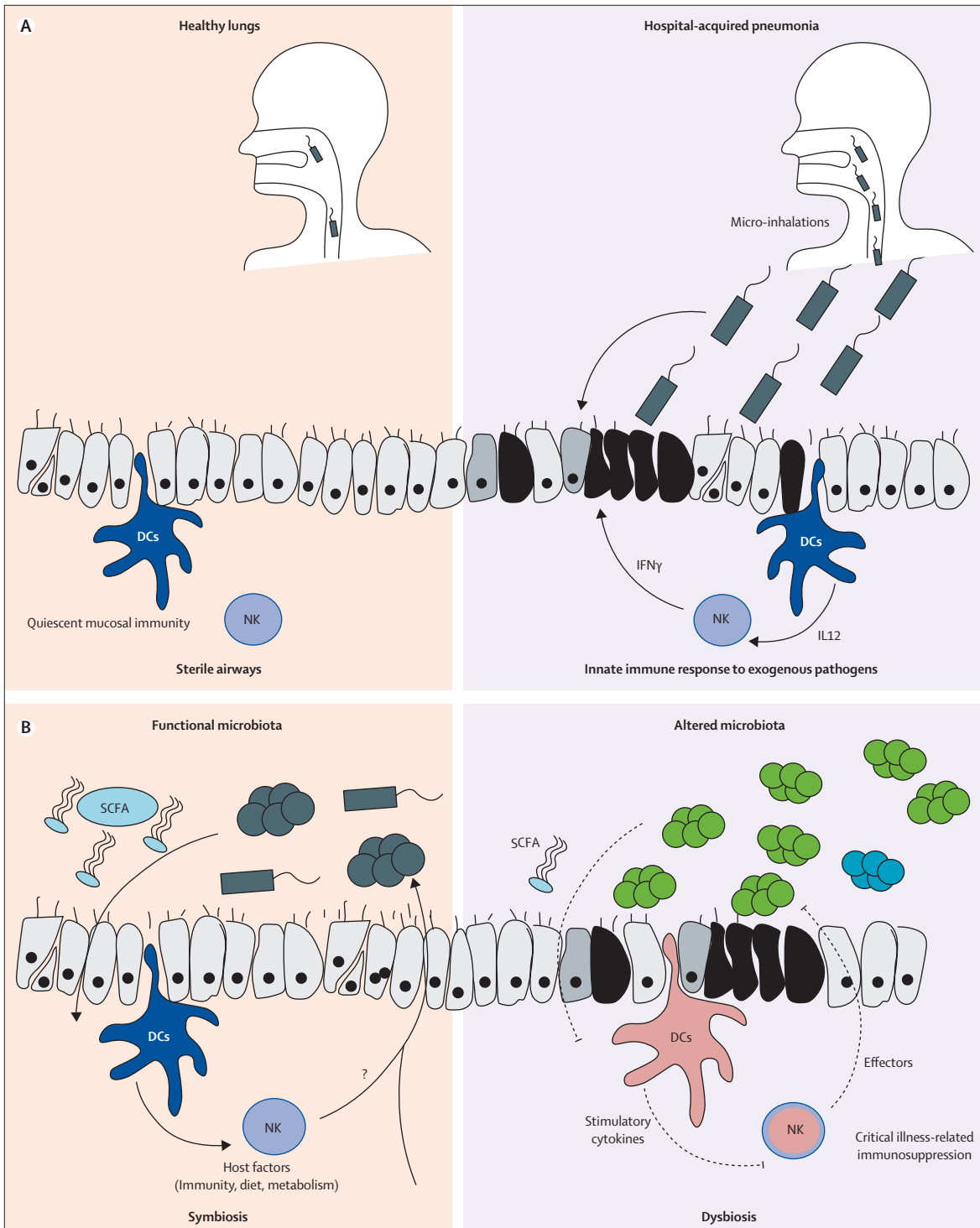


Figure 1: Physiopathology of hospital-acquired pneumonia

DC=dendritic cells. NK=natural killer cells. IFN=interferon. IL=interleukin. SCFA=short chain fatty acids. (A) Historical pathophysiological concept. Healthy distal airways are sterile (left). In response to the lung contamination by digestive bacteria, immune cells release cytokines which control the bacterial load but cause epithelial injuries. (B) Refined pathophysiological concept. In healthy lungs (left), the commensal derived metabolites induce tolerance by mucosal immunity. During hospital acquired pneumonia, the dysbiosis between microbiome and immunity results in epithelial injuries.

the ventilator promotes a specific histological pattern of pneumonia confirmed in post-mortem human studies.⁸ Therefore, health-care professionals should be cautious before applying the proposed concept of dysbiosis to the physiopathology of community-acquired pneumonia.

Limitations of the classic prevention method for hospital-acquired pneumonia

Healthy distal airways have long been considered sterile, and hospital-acquired pneumonia was thought to be caused by contamination of the lungs by exogenous pathogens, notably during microaspiration of digestive contents. This hypothesis, commonly termed the gastro-pulmonary route of infection, was proposed more than 20 years ago after evidence showed that bacterial colonisation of the oropharynx and trachea precede the development of hospital-acquired pneumonia in patients with critical illness (figure 1).⁹

Based on this physiopathology, numerous strategies were designed to prevent lung contamination from the digestive tract, and these strategies are recommended in Europe. Three main approaches have been evaluated: diminishing the micro-aspiration and macro-aspiration of digestive flora, reducing the volume of oropharyngeal secretions aspirated into the lungs, and inhibiting overgrowth or alterations in the microbiota of the oropharynx or larynx. However, the incidence of hospital-acquired pneumonia has not decreased substantially over recent decades and still routinely exceeds 10 cases per 100 patients with critical illness admitted to hospital.¹⁰ After more than three decades of clinical research aimed at preventing contamination of the lung tract by oropharyngeal bacteria (150 randomised clinical trials including more than 30 000 patients), most of the tested prevention strategies reduced the risk of hospital-acquired pneumonia but did not adequately improve amount of time spent in hospital or in-hospital deaths.¹¹ Moreover, evidence showing that only a minority of patients with tracheal microbial colonisation develop hospital-acquired pneumonia,¹² and that healthy lungs are colonised by a microbiome mainly composed of bacteria,¹³ suggest that the physiopathology of hospital-acquired pneumonia relies not only on aspirations, but also on other mechanisms. The prevention of hospital-acquired pneumonia via a gastro-pulmonary route is therefore effective, but requires improvements.

Finally, using new high-throughput technologies of microbial identification independent from culture, Dickson and colleagues¹⁴ confirmed that some patients with sepsis had a respiratory microbiome enriched with gut-associated bacteria and used animal models to show that many of the bacteria present in the lungs of critically ill patients do not arrive via the aerodigestive tract. These observations showed that gut microbiota enrichment is not a regular occurrence in patients admitted to hospital and that the mainstream theory of the gastro-pulmonary route of the infection is insufficient to fully understand

the complexity of the development of acute lung inflammation.

Limitations of the classic treatment methods for hospital-acquired pneumonia

Hospital-acquired pneumonia treatment recommendations suggest targeting taxa with the greater relative abundance found upon culture of lower respiratory fluids with antimicrobial therapy,⁴ mostly because clinicians' misleading interpretation of results from bacterial culture can potentially discriminate commensal and pathogenic bacteria. Recommendations suggest selecting the narrowest spectrum of antibiotics, selected on the basis of the minimal inhibitory concentration of antibiotic (measured on the dominant bacterial species found in respiratory fluids), while potentially resistant minority species are not considered during treatment. The limitations of this approach are emphasised by the results of randomised trials evaluating antimicrobial therapy, in which the proportion of clinical cure of hospital-acquired pneumonia does not usually exceed 70% in patients with critical illness.¹⁵ The emergence of resistant bacteria, the prevalence of which remains below 10% in most European countries,⁶ cannot explain the percentage of ineffective treatment cases, and the development of new broad-spectrum antibiotics has not increased rates of clinical cure.¹⁶ Optimisation of antimicrobial therapy is important but can no longer be expected to improve patient outcomes. For example, inadequate empiric antimicrobial therapy is not associated with outcomes of *Pseudomonas aeruginosa*-associated hospital-acquired pneumonia,¹⁷ and optimisation of the pharmacokinetics of antibiotics did not result in more patients being cured in randomised controlled trials that evaluated the dose adaptation to the blood levels measured during the treatment.¹⁸ Clinical cure (defined as the clinical resolution of hospital-acquired pneumonia) did not consistently occur with bacteriological cure (defined as the eradication of the dominant pathogen at the end of treatment).¹⁹

Altogether, these observations suggest the need for a reappraisal of the treatment approach for hospital-acquired pneumonia, because ignoring the bacteria that are in low abundance (beneath the culture detection threshold), or difficult to grow *in vitro*, and selecting antimicrobial therapy on the sole criteria of its ability to limit the multiplication of bacteria (minimal-inhibitory concentration), leads to severe pitfalls regarding patient outcomes. For instance, the evidence that nasopharyngeal bacterial species which exist in lower numbers than the threshold of detection by culture are associated with pneumonia outcomes in children with viral infection,²⁰ and that the lung microbiota composition is associated with the development of acute respiratory distress syndrome in critically ill patients with trauma,²¹ suggests that it is necessary to consider the entire microbiome for therapeutic interventions, rather than only the most

numerous species. Moreover, the association of the metabolomic functions of the microbiome (notably the enrichment of the sphingolipid metabolism pathway) with pneumonia outcomes places the focus on the quality,²² rather than only on the quantity, of respiratory bacterial content. These observations help to explain the observed high rate of hospital-acquired pneumonia treatment failure with antibiotics. At the time of hospital-acquired pneumonia diagnosis, the respiratory tract is challenged by a high burden of bacteria (high load), while a reduction in the diversity (Shanon index) of the microbiome is also observed.^{23,24} In these conditions, antimicrobial therapy not only eliminates the most abundant bacteria, but also further reduces microbial diversity,²⁵ paving the way for the same bacteria (relapse with the same potentially resistant pathogens) or some initially rare bacteria (recurrence of hospital-acquired pneumonia) to extend the lung inflammatory response at the end of the treatment. The effects of antibiotics on both the dominant communities and the bacterial microenvironment should be taken into consideration.

Host–microbe interactions in lung health: respiratory symbiosis

The lungs represent the body's largest surface with environmental exposure, with an alveolar and airway surface area that is double that of the gut and thirty times that of the skin.²⁶ This vast interface harbours a diverse and dynamic ecosystem of bacteria, defined largely by the balance of immigration (via subclinical microaspiration of pharyngeal microbiota²⁷) and elimination (via mucociliary clearance, cough, and innate and adaptive host defences).²⁸ Research advances in lung microbiology (the discovery and characterisation of the lung microbiome) have prompted a parallel evolution in the understanding of lung immunology in health: the concept of so-called immune tone in healthy lungs, calibrated locally via host–microbe interactions.^{29,30} Previous understanding of lung health was that the microbial status of the lungs was dichotomous (either sterile or infected). Nowadays, research has informed the understanding that healthy lungs represent a continuum of microbial burden, diversity, and community composition. Additionally, the immune status of the lungs was previously conceptualised dichotomously (uninflamed in health, inflamed in infection); however, the immune status of healthy lungs more closely resembles that of the gut: continuous, dynamic, and closely reflecting the local microbial milieu.

Several observations support this new conceptualisation of host–microbe interactions in healthy lungs. In animal models using healthy mice, variations in lung microbiota (both diversity and community composition) correlate strongly with variations in lung immunity (eg, lung concentrations of interleukin [IL]-1 α).^{29,31} Crucially, the immune tone of healthy animals strongly correlates with lung microbiota rather than with lower gut or oral microbiota, revealing the centrality of local (lung–lung)

interactions between the host and the microbiome. Although the purported gut–lung axis might partially contribute to the calibration of lung immunity, the importance of local host–microbe interactions within the respiratory tract should not be overlooked or understated. Notably, it is recognised that microbiota directly shape the host immune system in other organs,³² via the production of immunomodulatory compounds and metabolites, such as short-chain fatty acids (SCFA).³³ Microbiome and mucosal immunity shape one another, acting symbiotically in healthy individuals (figure 1).

The respiratory microbiome in patients with critical illness: a new organ failure

In patients with critical illness, every feature of the lung microbiome changes: microbial burden, microbial diversity, community composition, and the host's calibrated response to the microbiome composition. Causes and consequences of the rapid alterations of the lung microbiome in patients with critical illness are progressively delineated.

The key ecological forces that shape the lung microbiome—immigration, elimination, and relative reproduction rates of respiratory microbiota—are all altered in patients with critical illness.³⁴ Whereas healthy lungs are populated via a low-abundance, subclinical immigration of pharyngeal microbes via microaspiration, in patients with mechanical ventilation, the aspiration of pharyngeal microbes is accelerated.³⁵ Additionally, culture-independent studies have revealed enrichment of lower gastrointestinal tract bacteria in the lungs of patients with sepsis and acute respiratory distress syndrome,¹⁴ suggesting hematogenous or lymphatic translocation of gut bacteria as a mechanism of immigration to the lung and other end-organs in critical illness.³⁶ Enrichment of the lung microbiome with pharynx-associated microbiota has been shown to correlate with increased alveolar concentration of inflammatory cytokines (eg, IL-1 α), increased alveolar lymphocytes (especially IL-17-producing CD4 cells), and a distinct metabolomic profile.²⁹ Moreover the mechanisms of microbial elimination—cough, mucociliary clearance, and host immune defences—are frequently impaired in patients with critical illness, both intrinsically by the disease and by medical interventions (eg, sedation, paralysis, and corticosteroids).³⁴ Finally, growth conditions for disease-associated lung bacteria are altered in injured and inflamed lungs, with increased nutrient supply, inactivation of bactericidal surfactant, and enrichment with selectively growth-promoting host factors (eg, catecholamines,³¹ interferon (IFN)- γ , and other inflammatory cytokines).³⁷

In hospital-acquired pneumonia, these ecological perturbations could change the lung microbiome into a fragile ecosystem, with a relatively high biomass susceptible to catastrophic collapse via overgrowth by a dominant pathogen. The most prominent bacterial taxa in the healthy respiratory microbiome (eg, *Prevotella* spp.,

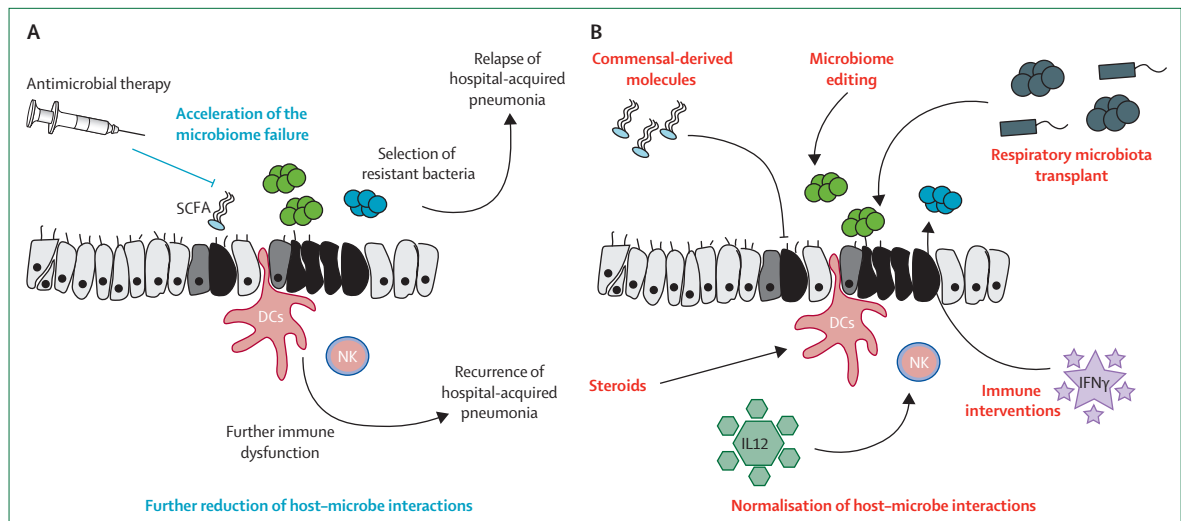


Figure 2: Current and proposed approaches to treat hospital-acquired pneumonia

DC=dendritic cells. NK=natural killer cells. IFN=interferon. IL=interleukin. SCFA=short chain fatty acids. (A) Antibiotherapy alters the metabolomic functions of the microbiome, selects resistant bacteria, and leads to relapse of recurrence of hospital-acquired pneumonia. (B) Normalisation of the microbiome and immune interventions have the potential to reduce inflammation and to clear virulent pathogens.

Veillonella spp.) become sparse with low abundance among patients admitted to hospital, with relative enrichment of familiar disease-associated taxa (eg, *Enterobacter* spp., *Staphylococcus* spp.).^{13,24,38} The biomass of the lung microbiome increases over time in patients with mechanical ventilation, and its diversity decreases before clinical development of hospital-acquired pneumonia.^{23,24} Ecologically, the onset of hospital-acquired pneumonia is characterised by a decrease in the diversity of the respiratory microbiome, potentially presenting as a collapse of a single species. Severity of illness is associated with changes in the microbiome of patients with sepsis.³⁹ An intrinsic challenge to the observational study of dysbiosis among patients admitted to hospital is the rampant issue of confounding exposures: patients in intensive care units are subject to innumerable influences that might directly or indirectly affect respiratory microbiota. These influencing factors include antibiotics, corticosteroids, sedation and supine positioning, and acid-suppressing medications, among others.

This disruption of lung microbiota might have major consequences for the host response during and after pneumonia. In children with viral pneumonia, microbial clusters (characterised by enrichment of bacteria beneath the threshold of detection by culture, such as *Haemophilus influenzae* or *Staphylococcus aureus*), are associated with immune response and the risk of admission to intensive care units.²⁰ In models of shock, germ-free mice are protected from the alveolar inflammation and injury seen in acute respiratory distress syndrome.⁴⁰ Furthermore, alterations in lung microbiota are significantly correlated with alveolar concentration of inflammatory mediators, such as tumour necrosis

factor- α , during acute respiratory distress syndrome and pneumonia.¹⁴ However, to what extent the microbiome's effects on lung immunity in pneumonia reflect the most abundant taxonomic group (usually the culture-identified pathogen), and to what extent they reflect the long tail of lower-abundance taxa detected in respiratory specimens, remains undetermined. Notably, Sulaiman and colleagues⁴¹ found that alveolar inflammation strongly correlates with microbiota signatures rather than with the presence of nontuberculous mycobacteria in patients diagnosed with nontuberculous mycobacterial disease. Moreover, an association between the loss of metabolic products released by the low-abundant bacteria and respiratory outcomes has been observed during viral bronchitis,²² but has not yet been thoroughly investigated for hospital-acquired pneumonia. The relative contributions of lung and gut microbiota in inducing acute inflammation during pneumonia and recovery remain undetermined. The classic paradigm of pharyngeal bacterial aspirations can be considered as one of the main causes, but not the sole cause, of perturbations of the respiratory microbiota in patients admitted to hospital, resulting in development of pneumonia.

Protection and modulation of the respiratory microbiome to prevent or treat hospital-acquired pneumonia

These observations suggest the microbiome plays a role in determining the severity of lung inflammation and injury in hospital-acquired pneumonia. Understanding whether the microbiome can be therapeutically manipulated (rather than eliminated by antibiotics) to prevent or resolve hospital-acquired pneumonia, or accelerate recovery following illness, is important (figure 2). Although the

microbiome is a therapeutic target for hospital-acquired pneumonia, clinical data are sparse, and our discussion must draw inferences from diseases other than hospital-acquired pneumonia, and microbiota outside of the lungs.

The re-establishment of normal microbiota after antibiotic treatment has the potential to prevent relapses of hospital-acquired pneumonia, notably those involving resistant pathogens, and to diminish lung inflammation during infections.⁴² Faecal microbiota transplant is highly effective in treating gut inflammation in patients with active ulcerative colitis⁴³ and preventing recurrent *Clostridium difficile* infection.⁴⁴ Repopulation of normal lung microbiota has not been attempted in humans, though experimental transfer of lung microbiota has been accomplished in animal models.⁴⁵ Given the contiguity of the upper and lower respiratory tracts, and their close communication in patients with critical illness, selectively repopulating pharyngeal microbiota might be more feasible and effective to normalise the respiratory microbiota.⁴⁶ The issue of building an industrial pharmaceutical process to produce so-called normal community transplant therapy would diminish the potential of widespread use,⁴⁷ contrary to the administration of probiotics, which can be standardised. Although several trials investigating probiotics have shown promise in humans with critical illness or risk factors for hospital-acquired pneumonia,⁴⁸ a large randomised controlled trial of patients with severe pancreatitis indicated increased risk for mortality in patients receiving probiotics.⁴⁹ Before probiotics can be used as a routine hospital-acquired pneumonia prevention strategy, certain limitations must be overcome. First, specific species must be identified with major immunological functions, which can be cultured *in vitro* and administered alone,⁵⁰ be capable of editing the lung microbiota by targeting the bacterial metabolism without eliminating the pathogen,⁵¹ and compensate for the failed production of commensal-derived molecules⁵² (figure 2). Second, biomarkers should be developed for the selection of patients who will respond to the oropharyngeal administration of probiotics (see Theranostic: biomarkers for patient stratification).

Critical illness-related immunosuppression

Lung mucosal immunity can either eliminate or tolerate commensal bacterial agents. The lung microenvironment is naturally highly tolerant to foreign bodies to avoid excessive inflammation in response to inhaled particles. The lung microbiome plays a key role in the immune tolerance of the lung microenvironment. Mucosal tolerance state is maintained by subpopulations of immune cells, essentially macrophages and dendritic cells.^{53,54} The transcriptomic activity of circulating leukocytes during hospital-acquired pneumonia, compared with community-acquired pneumonia, is characterised by overactivation of genes involved in cell remodelling and under-expression of type-I IFN signalling signature,⁵⁵ showing that the balance between immunogenic and

tolerogenic responses is altered in patients admitted to hospital.⁵⁶ As described in acute respiratory distress syndrome, or sepsis,^{57,58} two phenotypes of immune response to secondary infections can be observed: an increased reactivity of innate immunity (so-called trained immunity⁵⁹) or a state of tolerance.^{60,61} The physiological continuum between tolerance and trained immunity during secondary infections can be explained by the load of the pathogen in regulating the itaconate metabolic pathway.^{62,63}

Studies in patients with critical illness considered to be immunocompetent have revealed alterations in the function of several immune cells, notably myeloid cells (monocytes, macrophages, and dendritic cells), innate-like lymphocytes (natural killer, natural killer T cells), and conventional lymphocytes (B cells and T cells).⁶⁴ Furthermore, comparative analysis of the host response to community-acquired pneumonia and to hospital-acquired pneumonia in patients with critical illness has revealed distinct transcriptional and plasma protein responses,⁵⁵ showing the functional alterations of the immune response in patients admitted to hospital. These immune defects (so-called critical illness-related immunosuppression), are common features observed after all severe conditions associated with systemic inflammatory response syndrome, such as after major surgery, trauma, acute brain injury, or sepsis.^{56,65} Critical-illness related immunosuppression can be observed as early as after the first minutes of injury⁶⁶ and can persist for months. No causal link between these immune defects and the development of hospital-acquired pneumonia has yet been shown, but the risk of hospital-acquired pneumonia is directly correlated with the severity of these alterations: in particular with the decrease of Human Leukocyte Antigen-DR expression on the circulating monocytes (surrogate marker of a decreased antigenic presentation capacity),⁶⁷ with the circulating levels of immunosuppressive cytokines (such as IL-10),⁶⁸ and with the depletion of the T-lymphocyte repertoire.⁶⁹ Overall, the involvement of a state of critical illness-related immunosuppression in hospital-acquired pneumonia is now widely accepted, and some authors have proposed that the development of immune therapies that aim to restore immune function during critical-illness induced immunosuppression will reduce mortality of patients.^{56,70}

The mechanisms of these immune defects are numerous, and extensive reviews have been published.^{56,70} Patients with inherited deficiency in dendritic cells, IL-12, or IFN- γ are susceptible to respiratory infections.⁷¹⁻⁷³ The maturation defect of the *ex vivo* granulomatous response of patients with hospital-acquired pneumonia is characterised by a decreased recruitment of monocytes and natural killer cells, as opposed to regular recruitment of conventional lymphocytes.⁷⁴ For simplicity, and because myeloid cells (macrophages and dendritic cells) and natural killer (NK) cells are typical to the immune response involved in hospital-acquired pneumonia, we

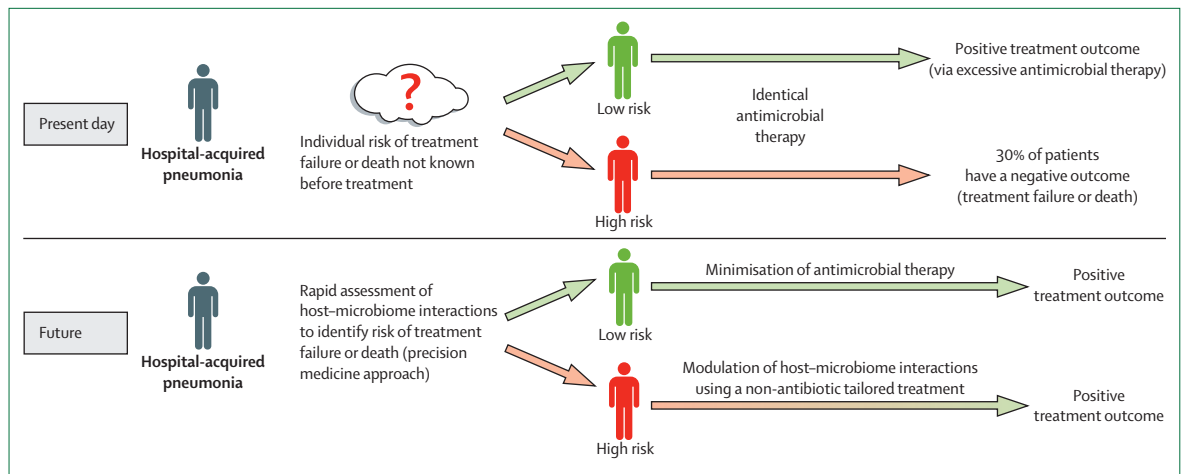


Figure 3: Expected outcomes of present day and future approaches to treatment of hospital-acquired pneumonia

Nowadays, patients with hospital-acquired pneumonia receive antimicrobial therapy with no consideration for their individual risk of treatment failure and mortality. Host-pathogen and microbiome interactions can be investigated in the future to stratify patients to develop precision medicine for hospital-acquired pneumonia.

will limit our description to the causes and the consequences of the defects of these cells in patients with hospital-acquired pneumonia.

During the initial systemic inflammatory response of any acute disease, the depletion in organs of immature ready-to-respond innate cells (such as immature dendritic cells), is a major determinant of immunosuppression.⁷⁵ Following an inflammatory process, modifications of the epigenetic regulation of myeloid cells⁷⁶ and local modulation of the terminal maturation programming of these cells⁶¹ are responsible for prolonged and potentially lifelong modifications in responses to infections. Additionally, metabolic defects in myeloid cells in patients with severe infections can lead to defects in their host defence function,⁷⁷ and are likely to contribute to increased susceptibility to hospital-acquired pneumonia.

The production of cytokines from dendritic cells is as important as antigen presentation in controlling the lymphocyte response to immune stimulation.⁷⁸ The production of IFN- γ from natural killer cells is important in the immune response to extracellular bacterial pneumonia, but the direct stimulation of natural killer cells by pathogens does not induce IFN- γ production.⁷⁹ The identification of bacteria by dendritic cells is required to trigger the production of factors such as IL-12 to stimulate natural killer cells, which in turn triggers natural killer cell licensing and IFN- γ secretion.⁷⁹ Although the cross-talk between dendritic cells and natural killer cells appears central in inducing the host response to infection,^{80,81} the failure of the IL-12/IFN- γ axis is commonly observed and associated with hospital-acquired pneumonia.

Perspectives: host-targeted approaches to prevent or treat hospital-acquired pneumonia

Based on these specific factors identified in the host response, (including decreased production of IL-12 or

IFN- γ) novel host-targeted approaches can be proposed to prevent and treat hospital-acquired pneumonia. Such interventions have the potential to address the problem of antimicrobial drug resistance, with patients admitted to hospital likely to receive less antimicrobial therapy (figure 3). The proof-of-concept of the efficiency of host-targeted treatment of hospital-acquired pneumonia is evident in randomised clinical trials showing that low doses of hydrocortisone reduce the risk of pneumonia in patients with trauma.^{82,83}

Decreased natural killer cell stimulation by myeloid cells contributes to the susceptibility to pneumonia in patients admitted to hospital, and reactivation of the IL-12/IFN- γ axis, as a compensatory therapy of critical-illness related immunosuppression, has the potential to restore immunity and enhance the treatment of hospital-acquired pneumonia. Direct injections of human recombinant IL-12 or of IFN- γ ,^{84,85} or the modulation of myeloid cells by Toll-like receptors⁸⁶ have been proposed as treatments for critical illness-related immunosuppression. These treatments have not yet shown their efficacy, but they appeared to be safe in patients in a phase 1 trial and case reports.^{84,85} Pilot randomised trials to test host-targeted approaches are required, but should be associated with the development and the validation of biomarkers to define responders and non-responders.

Other immune interventions targeting specific mediators involved in this condition, such as T regulatory cells or IL-7, should also be tested in the near future.⁶⁴ For example, as shown in trials involving the gut and skin, the editing of the respiratory microbiome has the potential to modulate dendritic cell function.^{87,88}

Theranostics: biomarkers for patient stratification

Each individual patient responds differently to therapeutic intervention. Therefore, patient stratification,

	Technical issues	Potential effects on treatment of hospital-acquired pneumonia
Determination of dominant taxa	Association between bacterial culture, microbiome composition, and immune response	Targeted bacteria under the threshold of culture
Determination of the metabolomic functions of the respiratory microbiome	Association of metabolomic functions of the microbiome with hospital-acquired pneumonia	Bacterial editing (rather than elimination)
Determination of the cross-talk between the bacterial and viral microbiomes	High throughput analyses of respiratory fluids	Introduction of biomarkers and phagotherapy
Determination of lung immunophenotypes before and during hospital-acquired pneumonia	Association between immune phenotype and hospital-acquired pneumonia (single cell RNAseq of peripheral blood mononuclear cells, high-dimensional single-cell mass cytometry)	Introduction of biomarkers; new potential host-targeted approaches
Description of lung subphenotypes of hospital-acquired pneumonia on the basis of host-pathogens interactions	Combination of high throughput analyses of microbiome and immunity	Precision medicine
Methods for <i>in vivo</i> modulation of host-pathogen interactions	Delivery of normal microbiota to the lungs; injection of immunomodulators	Non-antibiotic treatment of hospital-acquired pneumonia; Decrease of antimicrobial resistance

Table: Challenges and a research roadmap for treating hospital-acquired pneumonia

which predicts therapy responses, is urgently needed. No reliable biomarkers have yet been identified for the prediction of the course of hospital-acquired pneumonia, or for stratification of patients predicting therapy success, or degree of immunosuppression. However, several biomarkers have been associated with hospital-acquired pneumonia in patients with critical illness,⁶⁷ but none of these have been translated into clinical practice.⁴ Treatment of hospital-acquired pneumonia is adapted to the dominant pathogen in the respiratory fluid but this so-called one-size-fits-all approach, without adaptation to the individual, risks a large proportion of patients not responding to treatment.

Temporal alterations of the bacterial microbiome reflect variations in baseline lung innate immunity,³⁰ and the composition of the virome is associated with immunosuppression in organ transplant recipients.⁸⁹ Since the compositions of the bacterial and viral microbiomes are altered before the development of hospital-acquired pneumonia,²⁴ investigation of their evolution can be proposed as an *in vivo* test to understand the evolution of communities under treatment and to monitor the response to immunotherapy in patients with mechanical ventilation, for whom the respiratory airways can easily be accessed. In patients for whom the respiratory tract cannot easily be accessed, the temporal evolution of the circulating virome⁸⁹ or nasopharyngeal microbiome²⁰ could be tested as surrogate markers of the respiratory microbiome. The definition of subphenotypes of hospital-acquired pneumonia on the basis of the composition of the virome, microbiome, and transcriptomic host activity, will be a major deliverable of such approaches. If these high throughput analyses cannot be used at the bedside, the use of standardised rapid multiplex PCR panels for the diagnosis of severe infections shows that it is possible to implement rapid biomolecular analysis in acute cases⁹⁰ (figure 3).

Reliable stratification of patients into low risk or high risk of negative treatment outcomes and predictive biomarkers based on host immune status are still largely lacking. The main reason for this is the interperson variability of leukocyte populations (eg, widely varying frequencies of innate lymphocytes across individuals). Therefore, bulk-omics approaches do not effectively capture this complexity and only single-cell resolution can provide deeper insights into the dynamic immune responses or lack of response to treatment in hospital-acquired pneumonia. The new gold standard is unambiguous and unbiased characterisation of the myeloid system and lymphocyte compartment.⁹¹ Single cell RNA-transcriptomics⁹² or high-dimensional single-cell mass cytometry^{93,94} are available to capture the complexity of the immune response and can be used to stratify patients into a low or high risk of poor outcomes and into responders or non-responders to immunotherapy.⁹⁴ The key to this workflow is the use of large cohorts of patients, bar coding of the samples, and high-dimensional single-cell analysis followed by unbiased algorithm guided analysis.⁹¹ In future, research should aim to combine clinical factors (notably demographic data) and so-called omics characterisation of respiratory dysbiosis to develop clinicobiological scores with high accuracy for the stratification of patients. The integration of the host response and the microbiome composition has excellent accuracy in the diagnosis of lower respiratory tract infection in adults with critical illness, and shows the potential of protocols regarding investigation of host-pathogen interactions for the development of personalised treatments for hospital-acquired pneumonia.⁹⁵ Unsupervised analysis of high throughput methods (similar to those used for sepsis or acute respiratory distress syndrome)^{57,58} can describe specific phenotypes of hospital-acquired pneumonia, which can be used to develop a precision medicine approach (figure 3).

Search strategy and selection criteria

We searched the Cochrane Controlled Trials Register and PubMed (MEDLINE/Index Medicus) for studies published from January, 1969 up to January, 2019. Both human and animals studies were considered. The Medical Subject Heading terms used for the search were "pneumonia", "treatment", "microbiota", "mucosal immunity", and "biomarkers". No criteria were used for exclusion of studies in this review of literature.

Conclusions

We propose that hospital-acquired pneumonia is not an acute condition solely induced by the contamination of the respiratory tracts by exogenous pathogens, but the result of dysbiosis between the lung microbiome and mucosal immunity. This reappraisal of the physiopathology of the condition changes our understanding of the mechanisms involved in the genesis of hospital-acquired pneumonia and could lead to changes in prevention methods and treatment. The high proportion of ineffective treatment cases observed with antimicrobial therapy can be explained by a further reduction of bacterial diversity and metabolic functions within antibiotic treatment. The development and validation of strategies to restore the lost functions of the microbiome and of mucosal immunity will minimise—or even replace—antibiotics, which are the sole therapies used to date for the management of hospital-acquired pneumonia. Progress in high throughput analyses has enabled the development of approaches testing the host–pathogen interactions and the development of a precision medicine strategy (table). We propose that preventive methods and treatment of hospital-acquired pneumonia should aim to normalise the diversity of the microbiome and restore mucosal immunity, rather than to sterilise airways.

Contributors

AR, AT, RD, JAV, MGN, BB, and KA equally contributed to the literature search and data analysis. AR wrote the first draft of the manuscript and proposed the figures to all co-authors. AT, RD, JAV, MGN, BB, and KA extensively revised the manuscript. All authors have approved the final manuscript for publication.

Declaration of interests

A patent "Interleukin 12 for pneumonia" belongs to the University Hospital of Nantes (AR and KA employer) and to the University of Melbourne (JAV employer). All other authors declare no competing interests.

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