Human models of acute lung injury

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Acute lung injury (ALI) is a syndrome that is characterised by acute inflammation and tissue injury that affects normal gas exchange in the lungs. Hallmarks of ALI include dysfunction of the alveolar-capillary membrane resulting in increased vascular permeability, an influx of inflammatory cells into the lung and a local procoagulant state. Patients with ALI present with severe hypoxaemia and radiological evidence of bilateral pulmonary oedema. The syndrome has a mortality rate of approximately 35% and usually requires invasive mechanical ventilation. ALI can follow direct pulmonary insults, such as pneumonia, or occur indirectly as a result of blood-borne insults, commonly severe bacterial sepsis. Although animal models of ALI have been developed, none of them fully recapitulate the human disease. The differences between the human syndrome and the phenotype observed in animal models might, in part, explain why interventions that are successful in models have failed to translate into novel therapies. Improved animal models and the development of human in vivo and ex vivo models are therefore required. In this article, we consider the clinical features of ALI, discuss the limitations of current animal models and highlight how emerging human models of ALI might help to answer outstanding questions about this syndrome.

Acute lung injury: clinical perspective

Acute lung injury (ALI) and its more severe manifestation, acute respiratory distress syndrome (ARDS), are characterised by acute inflammation that affects the function of the gas exchange surface of the lung. The disorder affects all age groups, and has an incidence of approximately 200,000 cases per year in the United States and a mortality of around 35% (Rubenfeld et al., 2005). ARDS was first described by Ashbaugh and colleagues (Ashbaugh et al., 1967) in 1967 in a series of 12 patients. A working definition of ALI and ARDS was established in 1994 by the American European Consensus Conference (AECC) (Bernard et al., 1994). ARDS presents clinically with breathlessness and hypoxaemia in the presence of diffuse pulmonary oedema on the chest radiograph. Hydrostatic lung oedema due to elevation of left atrial pressure should either be clinically improbable or excluded by direct measurement or echocardiography for a diagnosis of ARDS to be confirmed. The severity of hypoxaemia distinguishes ALI from ARDS: when hypoxaemia is severe (partial arterial pressure of oxygen (PaO₂)/fractional concentration of oxygen in inspired air (FIO₂)<200 mmHg or 26.7 kPa), the disorder is termed ARDS, whereas less severe abnormalities of gas exchange (PaO₂/FIO₂<300 mmHg or 40 kPa) are termed ALI. In practice, ALI is an umbrella term used by the majority of epidemiological and interventional studies in this field and will be used in this article to encompass both clinical syndromes.

ARDS can result from both direct pulmonary injury, such as pneumonia, or indirect blood-borne insults – for example, following severe sepsis, a multiple-organ dysfunction syndrome (Ware, 2006). Risk of progression to ALI varies according to the type, number and severity of predisposing conditions, as well as the genetics and other characteristics of the patient (Marshall et al., 2002). The application of mechanical ventilation, which is required in up to 80% of patients, can further damage susceptible lung in a process termed ventilator-associated lung injury (VALI). The clinical impact of VALI was highlighted in The Acute Respiratory Distress Syndrome Network study, in which mortality was reduced from 39.8% to 31% with a low tidal volume ‘protective ventilator strategy’ (P=0.007) (A.R.D.S. Network, 2000). To date, the only strategy proven to be effective at reducing mortality in ALI is low tidal volume ventilation targeting a reduction in VALI. Although a recent Phase II study of neuromuscular blockade in early ALI demonstrated a reduction in 28-day mortality from 33.3% to 23.7% compared with placebo (P=0.05) (Papazian et al., 2010), this approach requires validation in further clinical trials.

Most ALI patients either die or are weaned from supportive treatment within 1-2 weeks, although up to 10% of patients require prolonged (30 days or more) mechanical ventilation. Lung function in most survivors returns to normal over 6-12 months. However, neuromuscular and psychological morbidity can significantly impair longer-term health-related quality of life (Hopkins et al., 1999; Herridge et al., 2003; Wilcox and Herridge, 2010).

Despite technological advances, for example in lung imaging, and significant investment in both basic science and large...
clinical trials, there are no lung-specific therapies that improve survival in ALI. This might be partly due to the difficulties associated with recapitulating a complex human syndrome in animal models. In this Clinical Puzzle, we highlight several outstanding questions regarding clinical ALI (Box 1) and discuss the limitations of currently available animal models. We then focus our discussion on how the development of human lung models, enabled in part by advances in clinical lung transplantation, might advance understanding of the pathogenesis of ALI and provide novel opportunities for therapeutic intervention.

**Pathophysiology of ALI**

The early phase of ALI is characterised by acute inflammation of the alveolar-capillary membrane, the cellular components of which include the alveolar epithelium, microvascular endothelium and specialised fibroblasts. The extent of alveolar epithelial damage is an important predictor of outcome (Matthay and Wiener-Kronish, 1990). Typical pathohistological appearances include extensive necrosis of alveolar type I cells and the formation of protein-rich hyaline membranes on a denuded basement membrane (Fig. 1). Loss of alveolar epithelial integrity results in the accumulation of protein-rich and highly cellular oedema fluid in the interstitium and alveoli. This inflammatory milieu consists predominantly of activated neutrophils and alveolar macrophages, which secrete inflammatory mediators that disrupt epithelial fluid transport and impair surfactant production by alveolar type II cells. Capillary thrombosis and extravascular fibrin deposition occur as a result of endothelial-dysfunction-associated upregulation and activation of tissue factor, and loss of the ability to activate the vitamin-K-dependent proteins C and S. This local pro-coagulant state potentiates pulmonary dysfunction and the acute inflammatory response (Idell, 2003; Bastarache et al., 2006; Wang et al., 2008). VALI exacerbates the syndrome further by physically disrupting or stimulating susceptible tissues and cells, resulting in the expression of pro-inflammatory and/or profibrotic mediators (Belperio et al., 2006). Pro-inflammatory mediators propagate lung injury and contribute to systemic inflammation, multiple-organ dysfunction and death associated with VALI. Following the acute inflammatory phase, most patients gradually recover normal physiology and lung function. In the alveolar epithelium, which is normally lined by approximately equal numbers of type I and type II cells, type II cells proliferate in response to stimulation by epithelial growth factors, including keratinocyte (FGF-7) and hepatocyte growth factors. Type II cells are also thought to act as progenitor cells for both daughter type II cells and type I cells (Sannes, 1991), differentiating into type I cells in response to specific developmental cues. These mechanisms combine to reconstitute the epithelial barrier and restore normal lung function (Sannes, 1984). However, if epithelial injury is severe or epithelial repair is impaired, a fibroproliferative phase of ALI can develop. During this phase, mesenchymal cells proliferate, neovascularisation occurs and the alveolar space

**Box 1: Outstanding questions in ALI research**

- What is the best approach for studying the complex interactions between the cells that populate the gas exchange surface of the lung? Specifically, what is the best way to study alveolar type I cell function and response to injury?
- Why do most survivors of acute lung injury (ALI) rapidly resolve the acute inflammation, whereas some progress to chronic persistent inflammation? How can we study lung repair mechanisms and therapies given that sequential analysis of distal lung tissue in patients is both ethically and technically challenging?
- What are the regulators of the fibrotic response after injury?
- How can we better translate experimental and animal-based studies into successful therapeutic targets for ALI?

**Fig. 1. Pathogenesis of ALI.** (A,B) Direct (e.g. pneumonia) or indirect (e.g. sepsis) injuries (A) result in sloughing of alveolar epithelial cells, causing the formation of protein-rich hyaline membranes (B). Neutrophils adhere to the activated capillary endothelium and migrate to the alveolus. Loss of alveolar-capillary membrane barrier integrity facilitates the accumulation of a neutrophilic inflammatory exudate in the interstitium and air space. Resident and recruited cells secrete inflammatory mediators that disrupt epithelial fluid transport and impair surfactant production by alveolar type II cells. Capillary microthrombosis and extravascular fibrin deposition potentiate pulmonary dysfunction and the acute inflammatory response. This interplay between inflammation and capillary thrombosis increases pulmonary dead space and shunt, contributing to the severe hypoxaemia observed clinically.
becomes filled with activated fibroblasts and myofibroblasts that synthesise excessive collagenous extracellular matrix. A small proportion of patients progress to a chronic phase of respiratory insufficiency characterised by widespread pulmonary fibrosis or scarring, with disordered lung architecture and evidence of subpleural and intrapulmonary cysts (Gattinoni et al., 1994).

**Animal models of ALI and their limitations**

Animal models are invaluable in the study of complex diseases such as ALI, allowing testing of hypotheses in complex biological systems. Rodent ALI models, in particular, have revealed important biological mechanisms underlying the temporal sequence of injury, inflammation and dysregulated repair.

Animal models of ALI can be divided into two main groups. Models that fall into the first group involve: (1) direct injury of the lung by noxious stimuli, including intrathoracic or intranasal administration of bacteria or bacterial products such as lipopolysaccharide (LPS) to model community-acquired pneumonia (CAP); (2) the administration of intratracheal acid to reproduce aspiration pneumonitis; or (3) simulation of VALI using high tidal volume ventilation. Models in the second group involve indirect lung injury, including: (1) sepsis models such as caecal ligation and puncture (CLP); (2) the administration of intravenous bacteria or LPS; and (3) the oleic acid model, which simulates the release of bone marrow oleic acid in ALI patients following long-bone fracture.

The injury in these models targets either the capillary endothelium or alveolar epithelium (Matute-Bello et al., 2008). Although each model can reproduce components of the key pathological changes that occur in ALI, none is capable of fully recapitulating the classical triad of features observed in patients: severe neutrophilic alveolitis with deposition of hyaline membranes and the formation of microthrombi. For example, although LPS administration elicits a neutrophilic inflammatory response with an increase in intrapulmonary cytokines, changes in alveolar-capillary permeability are mild (Wiener-Kronish et al., 1991). In fact, of the currently available animal models, only acid aspiration, CLP and VALI models mimic human aetiologies of ALI to a reasonable extent. Combination models using two mechanisms of injury, such as surfactant depletion and high tidal volume ventilation, might overcome some of these failings. Recapitulating the coagulation and fibrinolytic abnormalities of ALI, in particular, continues to pose a scientific challenge.

Despite extensive study in current animal models, none of the pharmacological interventions developed [including anti-tumour necrosis factor-α (TNFα) therapy, surfactant replacement and steroid treatment] has translated into measurable clinical benefit. Additional limitations might therefore exist – for example, there are fundamental differences in anatomy and physiology between humans and both small and large animals. Indeed, comparative proteomic analysis between bronchoalveolar lavage fluid (BALF) from an ALI patient and a mouse model of ALI identified only 21 homologous proteins (Gharib et al., 2010). In addition, airway architecture and pleural anatomy show marked differences (Albertine et al., 1984; Shapiro, 2006; Rock et al., 2010), and normal rates of alveolar epithelial fluid transport differ markedly across species (Matthy et al., 1985; Smedira et al., 1991; Sakuma et al., 1997). Primate studies are more closely representative of human disease but are logistically complicated, financially prohibitive, and fewer genetic and genomic tools are available for use in primates. A further drawback of animal models is interspecies difference in host immune response to epithelial injury and the coagulopathy of ALI. Animal size places limitations on sampling volume of, for example, blood and BALF, as well as in obtaining accurate physiological measurements. Modelling of established, severe ALI presents logistical problems in the provision of prolonged intensive care to critically ill rodents; most animals die from shock or hypoxaemia. Finally, as highlighted

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**Box 2: Human lung repair**

Under normal circumstances, adult mammalian lung is largely quiescent, with little cell turnover. However, following injury such as that described in the Case study, the lung has a dynamic but poorly understood capacity for repair. Recent advances in epithelial cell biology have identified candidate molecular factors and progenitor cell populations required for lung repair by exploiting the wealth of molecular biological and genetic techniques available in mouse models (Rawlins et al., 2007; Warburton et al., 2008; Crosby and Waters, 2010). The study of lung repair following ALI requires sequential sampling of distal, alveolar tissue, which is ethically and technically challenging in patients. The development of ex vivo lung perfusion (E Vi LP) models will allow intact human lungs containing large, thin and fragile type I epithelial cells to be maintained. Such models might provide a tractable system to study the cellular and molecular mechanisms underlying lung repair and to investigate strategies to augment this process in ALI patients.
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by Bastarache (Bastarache and Blackwell, 2009), there are no robust biomarkers in animal models of ALI that translate into the human syndrome, and vice versa.

Despite these limitations, animal models will continue to have a considerable role in the study of ALI, because few alternatives allow investigation and therapeutic testing in a complex biological system. The ‘ideal’ animal model of ALI is unlikely to be realised for many years. Until then, investigators will employ the model that best reproduces the pathological features tested by their hypothesis.

Human models of ALI

Where possible, human models of ALI (Table 1) should be used to develop and test novel therapeutic targets. In this section, we describe human in vivo models that will allow the testing of novel therapeutic compounds in Phase II proof-of-principle clinical studies, as well as describing human ex vivo models that might help to shed light on the pathogenesis of ALI (Fig. 2).

In vivo models

Surgical models

One-lung ventilation (OLV) is a technique used in thoracic anaesthesia and involves ventilation of a single lung, allowing the other one to collapse. The method optimises surgical access during lung resection and oesophagectomy procedures. OLV is associated with pulmonary inflammation, reflecting what is defined as subclinical ALI in most patients (Moloney et al., 2004) (Table 1). The majority of the pathophysiological features of OLV-induced ALI, including endothelial and epithelial dysfunction, neutrophil infiltration, and vascular congestion, are common to ALI of other aetiologies (Carden and Granger, 2000; Jordan et al., 2000; Her and Mandy, 2004; Yin et al., 2007). The incidence of ALI after lung resection is 2-4% after lobectomy and up to 8% after pneumonectomy, with a mortality of 40-60% (Kutlu et al., 2000; Ruffini et al., 2001; Dulu et al., 2006; Alam et al., 2007). The incidence of ALI after oesophagectomy in a single-centre study was 23.8% (Tandon et al., 2001). Pulmonary dysfunction following cardiopulmonary bypass (CPB) is common, with ALI occurring in 2% of patients. Causative mechanisms include ischaemia-reperfusion, endothelial dysfunction, systemic inflammation and atelectasis (Dodd-o et al., 2004; Syed et al., 2004; Fan et al., 2006). Analysis of intra-operative lung specimens showed evidence of alveolar oedema, extravasation of erythrocytes and neutrophils, and congested alveolar capillaries (Wasowicz et al., 1999). Electron microscopy demonstrated swollen and necrotic pneumocytes and endothelial cells (Wasowicz et al., 1999).

Given the elective nature of the surgery, these patients represent a cohort of potentially predictable ALI patients for future study. Consent for trials of either prophylactic therapy or disease-modifying therapy could therefore be undertaken pre-operatively. Owing to the relatively high associated incidence of ALI following oesophagectomy, patients undergoing this procedure could be a particularly useful cohort in which to carry out such a pharmacological study with a clinical as well as a surrogate endpoint. Indeed, a clinical trial of hydroxymethylglutaryl-CoA (HMGCoA)-reductase inhibitors to reduce post-oesophagectomy ALI is currently recruiting patients (http://www.controlled-trials.com/ISRCTN56543987).

Inhaled LPS

Inhalation of LPS, a component of the outer membrane of Gram-negative bacteria, results in a local subclinical alveolar inflammatory response with neutrophil recruitment and an increase in cytokine and chemokine production (Sandstrom et al., 1994; Jagielo et al., 1996; O’Grady et al., 2001; Nick et al., 2004), analogous to that which occurs in ALI (Table 1). Notably, the coagulopathic and fibrinolytic abnormalities associated with ALI are also replicated in the inhaled LPS model (Maris et al., 2005). Although a mild systemic inflammatory response is also observed, neither the pulmonary or systemic effects have been associated with significant adverse clinical events.

HMGCoA-reductase inhibitors have shown promise as an anti-inflammatory therapy in both in vitro and animal models of ALI (Nagashima et al., 2002; Jacobson et al., 2004; Fessler et al., 2005). On the basis of these data, inhaled LPS was administered to 30 healthy volunteers with or without pre-treatment with the HMGCoA-reductase inhibitor simvastatin. Compared with untreated controls, the group treated with simvastatin demonstrated a reduction in neutrophil numbers and activity in the alveolar space, and a reduction in cytokines in BALF (Shyamsundar et al., 2009). This study was the first to investigate the effects of a statin in vivo on LPS-induced pulmonary inflammation in humans, and provides proof-of-principle evidence supporting the beneficial anti-inflammatory effects of
statins in human lung. These data led to the initiation of a Phase IIb placebo-controlled trial of statins to treat established ALI (Craig et al., 2010). The statin-treated group showed improvements in non-pulmonary organ dysfunction. In addition, modest improvements in pulmonary function were observed in patients who were still ventilated at 14 days. This was associated with an early reduction in pulmonary and systemic markers of inflammation.

**Limitations of in vivo human models**

There are several pathological, ethical and logistical challenges that limit the use of in vivo surgical models in humans. First, these are short-term models of low-level inflammation: although many of the pathological hallmarks of ALI are re-created, the physiological decrements in gas exchange and the levels of proinflammatory cytokines induced are generally modest. Second, in vivo studies are inevitably constrained by clinical priorities and ethical considerations; healthy subjects enrolled in such studies should be thoroughly informed about potential risks. Third, it is important to recognise that the pathogenesis of ALI after operations involving both OLV and CPB is multifactorial, and patient factors combine with the direct effects of OLV and CPB to compromise pulmonary function in the early post-operative phase. Finally, studies involving healthy human volunteers mandate that patient safety is a priority; despite an established safety profile, inhaled LPS studies should be undertaken in settings where there is adequate medical supervision.

**Ex vivo models**

**Ex vivo perfused human lung models of ALI**

The development of ex vivo human lung models of ALI could offer a new paradigm in ALI translational research (Curtis et al., 2008). Such models might allow recapitulation of severe ALI, as well as experimental manipulation and temporal analysis of distal lung tissue, which are not achievable using in vivo human models.

Ex vivo lung research is not a new concept, and has been widely used to study lung function in small animals (Wang et al., 1989; Wisser et al., 1993; Broccard et al., 2002). Human ex vivo research uses consented donor organs that are unsuitable for transplantation based on International Society for Heart and Lung Transplant (ISHLT) criteria (Orens et al., 2006). Organs are maintained in a viable state with established
Human ex vivo models of ALI involve similar noxious stimuli to those used in animals, including the use of live bacteria, which can be either infused into lung tissue via the perfused vasculature or delivered to the airways and distal lung by nebulisation, or delivered topically under direct vision to a targeted area using a bronchoscope (Table 1). Such models could also allow the testing of disease-modifying therapies in ALI to generate relevant, reliable and predictable human pharmacodynamic, pharmacokinetic and toxicology data through analysis at the organ, cell, genomic and molecular level, before early phase clinical studies. Ex vivo modelling is superior to tissue- and cell-based assays because the architectural integrity of the lung is preserved. For example, type I pneumocytes, which cover over 90% of the gas exchange surface of the lung, are difficult to culture in vitro; therefore, little is known about the response of this cell type to injury and the subsequent mechanisms of repair.

Currently, there are two main techniques for maintaining lungs ex vivo: the isolated perfused lung model and the ex vivo lung perfusion (EVLP) model. In the isolated perfused lung model, the lungs, together with the heart, are surgically removed and suspended by the trachea in a humidified, jacketed chamber maintained at 36-37°C. The lungs are perfused with a buffer solution that enters the lung by the pulmonary artery and drains via the pulmonary vein. The buffer solution is then collected or re-circulated. The lung is either left unventilated, or continuous positive airway pressure is applied (Fig. 3).

Several recent studies have shown the potential of the isolated perfused lung model for studying the pathophysiology of ALI. In animal models, an increase in alveolar fluid clearance (AFC) results in less pulmonary oedema and a more rapid improvement in gas exchange during ALI (Saldias et al., 2000; McAuley et al., 2004; Frank et al., 2005). Clinical data suggest that intact AFC is associated with improved survival in patients with ALI (Ware and Matthay, 2001). Extrapulating these data, a model of ALI-associated pulmonary oedema was investigated in isolated perfused human lungs by a group from the University of California San Francisco, who found that β2- adrenergic agonists increased AFC (Frank et
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al., 2007). In addition, the study identified that the airspace and perfusate levels of the soluble receptor for advanced glycation end products (RAGE; a marker of alveolar type I epithelial cell injury) were elevated in lungs that did not have measurable AFC (<3%/hour) compared with those that did. Subsequently, a study of 30 ex vivo lungs showed that the rate of AFC was inversely correlated with RAGE levels in distal airway aspirate samples (Briot et al., 2009).

The same investigators then established a more refined model of ALI in their isolated perfused lung model (Lee et al., 2009). The right middle lobe or left lower lobe was perfused lung model (Lee et al., 2009). The translational potential of the model was realised through studying the effect of mesenchymal stem cells (MSCs) on AFC in the injured lobe over a period of 4 hours: MSCs reduced endotoxin-induced injury and inflammation and augmented AFC, compared with controls. Thus, the isolated perfused lung model represents a (short-term) translational model of ALI that reproduces some of the physiological, immunological and pathological features of human in vivo ALI.

EVLP was developed by Steen (Steen et al., 2003) as a method to assess the quality of donor lungs from non-heart-beating donors. It is used clinically to facilitate reconditioning and subsequent transplantation of unacceptable donor lungs (Egan et al., 2006; Wierup et al., 2006; Steen et al., 2007). In EVLP, an elaborate and expensive extracorporeal circuit and ventilator system replicate in vivo physiology (Fig. 3); lungs are ventilated with a low tidal volume strategy while a closed extracorporeal circuit with a centrifugal pump targets a pulmonary perfusate flow of 40-100% of the donor’s predicted cardiac output. An albumin-based perfusate is used to optimise oncotic pressure and reduce endogenous lung oedema. Whole lungs have been maintained on EVLP for up to 12 hours with preservation of lung function and avoidance of additional lung injury (Cypel et al., 2008; Cypel et al., 2009). Although ALI modelling in the EVLP set up has yet to be established, we propose that ALI models could be readily achieved based on the strategy used in the isolated perfused lung model.

Owing to their relatively prolonged viability, EVLP models offer the potential to study late-phase injury and, potentially, early repair in whole human lung. Sampling and subsequent cellular, molecular, proteomic and genomic analyses can take place hourly in a variety of formats, including peripheral and endobronchial lung biopsies, bronchoalveolar lavage (BAL) and perfusate, to generate large datasets. Moreover, serial imaging with, for example, computed tomography, can be undertaken, allowing unique structural and functional correlations in the distal lung.

**Limitations of ex vivo human models**

Although ex vivo modelling has many potential benefits, there are several limitations. It is important to recognise that most lungs donated for research have been declined for transplant: baseline physiology is often abnormal and there will inevitably be a degree of background ALI present prior to use of the tissue for experimental modelling. Moreover, donated lungs are unlikely to be homogeneous, with a wide variation in ischaemic times and donor comorbidities, including smoking. The direct impact of cold preservation of the lungs (for transport) on the development of ALI remains poorly understood. Available donor lungs are, by necessity, at a premium. Organ procurement is further limited by legal, ethical and cultural considerations, which vary between states and countries. This process is facilitated by collaboration with donor networks and organ procurement organisations. The fact that renal and hepatic metabolism are absent in ex vivo models also needs to be taken into consideration when interpreting pharmacokinetic data. Finally, the EVLP technique in particular requires a large amount of technical expertise from both surgeons and perfusionists, meaning that this type of model is logistically challenging to set up unless in a centre with cardiothoracic transplant expertise.

**Conclusions and future prospects**

Despite over 30 years of promising preclinical studies, no pharmacological intervention developed thus far has reduced mortality in this devastating syndrome. This fact alone highlights the limitations of current animal models. Human models of ALI are advantageous in that they allow potential therapies to be investigated in vivo in humans, hopefully providing more accurate information to inform subsequent larger clinical trials. Refinement and development of human ex vivo models of ALI will generate relevant, robust data prior to testing therapeutic agents in clinical trials on patients with ALI. The use of viable, structurally intact human lung for experiments might bypass many failings of current drug development platforms and allow unique opportunities to study the human alveolar-capillary unit in an appropriate microenvironment. Through collaboration between basic scientists, physiologists and clinicians, ex vivo modelling has the potential to become the gold-standard for preclinical testing of therapeutics for ALI.

**ACKNOWLEDGEMENTS**

This work was funded and supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, The National Institute for Health Research or the Department of Health.

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