syndrome) of whom 1084 (84%) were treated with methylprednisolone while 207 (16%) received no steroid treatment.[2] Glucose levels were the same at baseline in both groups but in those treated with steroid the mean value rose significantly. The highest blood glucose in the methyprednisolone group was 8.68 mmol/l (±4.8) compared with 6.39 mmol/l (±3.71) in the non-steroid cohort ($p<0.05$).[2] This change is comparable with the 1.8 mmol/l increase observed with hydrocortisone in a multicentre randomised trial of steroids in sepsis.[3]

An increase of this magnitude appears trivial, but significantly alters glucose levels within the lung. Airway surface fluid is a key element of pulmonary defence, and glucose is normally maintained 3–20 times lower than plasma levels by active transport mechanisms.[4] The latter has a threshold of 6.7–9.7 mmol/l and glucose increases in airway fluid when plasma levels exceed this value. Furthermore, pulmonary inflammation disrupts epithelial integrity and also leads to a rise in lung glucose. Airway surface fluid contains surfactant proteins A and D, which not only are important host defence molecules against a broad spectrum of pathogens but, in addition, possess a number of immunoregulatory properties. These proteins are members of the collectin family, which recognise carbohydrate moieties on microorganisms through their lectin domain. The latter also binds glucose, which may act as a competitive inhibitor of surfactant proteins.[5] It is little surprise, therefore, that raised airway fluid glucose promotes pulmonary inflammation and infection.[4]

Corticosteroids are an important treatment modality in many pulmonary and extrapulmonary diseases. It is likely that in many diseases such as COPD, interstitial lung disease and asthma, modest hyperglycaemia associated with steroid use abrogrates the beneficial anti-inflammatory effects of these drugs. Further investigation of this phenomenon is warranted not only in COPD, but also in other pulmonary diseases in which steroids are commonly used.

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## Authors' response

We thank Dr Wise and colleagues[1] for their thoughtful response to our work in chronic obstructive pulmonary disease patients with decompensated hypercapnic respiratory failure.[2] We believe that modest hyperglycaemia is a useful way of identifying patients at greatest risk of treatment failure with non-invasive ventilation, but we are more cautious than those correspondents in implicating corticosteroid use either acute or chronic as a major aetiological factor. Our study was clearly underpowered to exclude such an association but we did not see any trend towards a worse outcome in relationship to previous oral corticosteroid use. The issues reported in the patients with severe acute respiratory syndrome taking methylprednisolone are less likely to apply in our patients in whom the dose of systemic corticosteroids used to treat chronic obstructive pulmonary disease exacerbations is significantly lower than in the severe acute respiratory syndrome study or than that reported in the USA.[3 4] Previous use of inhaled corticosteroids can be associated with clinically diagnosed pneumonia, but hyperglycaemia was not an issue in that large trial nor is pneumonia incidence always increased by inhaled steroid use.[5 6] The mechanisms suggested by which hyperglycaemia promotes lung infection are plausible but will be difficult to test in humans. Disappointingly, recent data suggest that tightly controlling hyperglycaemia in an intensive care unit setting is associated with worse rather than better outcomes, which support our view that this may be a marker of disease severity rather than a causal factor leading to a worse outcome.[7]

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## *Pneumocystis jirovecii* in pleural infection: a nucleic acid amplification study

Pleural infection is associated with 20% mortality in the 80 000 new cases per year in the UK and USA. *Streptococcus* species cause ~50% of community-acquired bacterial pleural infection.[1] *Staphylococcus aureus* and anaerobes are isolated in 8% and 20% of cases, respectively, and 12% of pleural infections yield polymicrobial cultures. However, even using culture and nucleic acid amplification techniques (NAATs), 26% of cases remain microbiologically obscure.

The negative microbiology may be due to previous antibiotic treatment, varying pathogen prevalence in different pleural fluid locules (already known to vary biochemically[2]) or the presence of organisms that are difficult to detect using conventional techniques. One such possible organism is *Pneumocystis jirovecii*, which requires specialist diagnostic techniques (eg, Grocott–Gomori methenamine silver staining or NAATs).

*P jirovecii* has been identified in sputum and bronchoalveolar lavage (BAL) fluid from both immunocompromised and immunocompetent individuals—it has been isolated from BAL fluid using NAATs in 18% of patients with lung disease without HIV

undergoing bronchoscopy,[3] in BAL fluid from 4.4% of general medical patients with community-acquired bacterial pneumonia[4] and in the oropharyngeal washes of 20% of a healthy population.[5] In pleural fluid, however, *P jirovecii* has only been studied and reported in those immunocompromised with HIV.[6] There has been, to date, no systematic examination for *P jirovecii* in pleural fluid.

Given the prevalence of *P jirovecii* in chronic lung disease and asymptomatic healthy people, we hypothesised that it might be a passenger or co-pathogen in infected pleural fluid.

We assessed the prevalence of *P jirovecii* in 133 samples of pleural fluid from 126 patients with established pleural infection, using a *P jirovecii*-specific NAAT. Table 1 shows the clinical and laboratory characteristics of the participants.

A probe-based quantitative PCR technique was used, targeting the *P jirovecii* heat shock protein 70 (HSP70) gene to detect and quantify the presence of *P jirovecii* DNA.[7] Both positive and negative controls were included. Assessment of inhibition was made using spiked linearised HSP70 *P jirovecii* plasmids.

There was no evidence of *P jirovecii* DNA in any of the pleural fluid samples. Two pleural fluid samples showed evidence of inhibition of the PCR; a 2.71 increase in $C_q$ (quantification cycle) in one patient and a 4.68 increase in $C_q$ in the other.

Absence of *P jirovecii* in the pleural space, despite its prevalence in the lung, is particularly interesting. This may be due to its tropism for the lung, where it exists primarily as an alveolar pathogen (adherent to glycoprotein A on type 1 alveolar cells), usually without host invasion. Such attachment to alveolar cells may be a requirement for proliferation; perhaps the avidity of *P jirovecii* for alveolar cells makes it unable to reproduce in the pleural space without overwhelming immunosuppression. Limited capacity to bind to the cell surface of mesothelial cells of the visceral pleura may also prevent *P jirovecii* from entering the pleural space.

Our study also investigated the influence of co-purified inhibitors on the PCR, essential for accurate assessment of the specific nucleic acid within the sample. Importantly, we found that 1.5% of pleural nucleic acid extracts showed minor inhibition of PCR. This inhibition may be due to a high concentration of host genomic DNA released from lysed neutrophils, a characteristic finding in pleural infection (although extraction reagents and biological agents (such as immunoglobulin G and haemoglobin) may also cause inhibition). This finding has a clear relevance for future NAAT studies of infected pleural fluid—careful consideration must be given to the choice of nucleic acid extraction method. Inhibition assessment is essential if negative findings are to be reported with any confidence.

The absence of *P jirovecii* in pleural fluid in our large cohort of cases of typical pleural infection suggests that there is no need to perform routine investigations for *P jirovecii* in pleural infection unless a patient is severely immunocompromised.

**Table 1** Characteristics of participants (n=126)

| | |
|---|---|
| Age, years, median (IQR) | 56.0 (38.7–71.4) |
| Male, n (%) | 89 (71) |
| Duration of symptoms prior to presentation, median (IQR) | 14 (7–28) |
| Co-morbidity, n (%) | 86 (68) |
| Chronic respiratory disease | 15 (12) |
| Excess alcohol consumption | 17 (14) |
| Diabetes mellitus | 13 (10) |
| Patients with neutropenia on admission blood tests | 0 (0) |
| Pleural fluid characteristics | |
| Visibly purulent, n (%) | 104 (83) |
| Positive standard microbiology, n (%) | 69 (55) |
| *Streptococcus pneumoniae* | 12 |
| Anginosus group of streptococci | 13 |
| Other streptococci | 5 |
| *Staphylococcus aureus* | 9 |
| Anaerobic or mixed aerobic/anaerobic infection | 16 |
| Mixed aerobic bacteria | 8 |
| Gram-negative bacteria | 6 |
| pH in patients without frankly purulent fluid, median (IQR) | 6.9 (6.7–7.1) |
| Glucose (mg/dl), median (IQR) | 18 (11–61) |
| Lactate dehydrogenase (IU/l), median (IQR) | 6000 (1629–20000) |
| Patient laboratory characteristics | |
| Total white cell count ($\times 10^9$/l), median (IQR) | 14.7 (10.3–22.0) |
| C-reactive protein (mg/l), median (IQR) | 187 (83–271) |
| Albumin (g/l), median (IQR) | 27 (22–31) |

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**Ethics approval** This study was conducted with the approval of the Anglia and Oxford Multicentre Research Ethics Committee (MREC) (ref: 98/5/61), the Oxfordshire Research Ethics Committee (05/Q1605) and the Cambridgeshire Research Ethics Committee (04/MRE05/53).

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