deliver a definitive trial examining the efficacy of HFCWO.

There is unlikely ever to be a perfect airway clearance regime. Decisions regarding the optimal airway clearance regime for patients are not easy, and are complicated by the various clinical phenotypes of patients with CF and the number of options available. It is probable that all airway clearance regimes work by different mechanisms to enhance airflow and reduce mucus viscosity, both of which are important for optimal airway clearance.¹⁹ Before new devices become available, research needs to provide clear evidence that the new device can achieve enhanced airway flow and changes in viscosity required for optimal airway clearance. Research also needs to focus on the role of devices in specific subgroups of patients (eg, patients with large sputum volumes or non-productive of sputum) or in specific situations (eg, in stable disease or during an exacerbation). There is a high demand on patients with CF to take part in both pharmaceutical and non-pharmaceutical studies so, in order for these physiotherapy trials to be prioritised in terms of funding and patient recruitment, the physiotherapy community needs consensus on what is the best study design to provide these sources of evidence, what sample sizes are required, how long these studies need to be and which outcome measures are appropriate. Lung function has been traditionally accepted as the primary outcome in airway clearance trials. However, the rate of change in lung function is slowing so much and is now as low as 1%, so it is unlikely that future airway clearance trials will be able to show any clear benefit in terms of lung function. This has been highlighted in a recent European Cystic Fibrosis Society (ECFS) consensus conference report on clinical trials which stated that new alternative outcomes need to be used.²⁰ Physiological measures such as lung clearance

index, cough monitors and sputum viscosity show promise as outcome measures, although consensus is needed on standardising the methodologies for these outcomes. Data are also needed on what magnitude of change is needed in these outcomes to translate into an important change in a clinical outcome. More emphasis needs to be put on how best to capture patients' experiences of different treatments in trials (both positive and negative), and data on adherence will be particularly important.

In conclusion, appropriately designed trials of adequate size using new alternative outcomes will ensure that future airway clearance trials provide us with the information we need to make informed decisions on the options for effective airways clearance techniques.

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Novel pulmonary biomarkers in the diagnosis of VAP

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Ventilator-associated pneumonia (VAP) is reported to occur in up to 20–27% of mechanically ventilated patients, and impacts healthcare in terms of patient morbidity, mortality and expenditure.^{1–3} The concept of VAP seems straightforward

ward—that is, alveolar inflammation due to an infectious agent that was not present at the time of initiation of mechanical ventilation. However, the diagnosis remains difficult. Importantly, this difficulty in diagnosis of VAP leads to potential over-/underprescription of antibiotics and misguided treatment.

The American Thoracic Society guidelines of 2005³ suggest that the use of readily available clinical data is adequate to inform the diagnosis of VAP. However, while such an approach has the advantage of being straightforward to apply, when compared with postmortem histological specimens the resultant sensitivity and

specificity were 69% and 75%, respectively,⁴ indicating the need for better diagnostic systems. More complex scoring systems combining clinical and microbiological data, for example the clinical pulmonary infection score⁵ and national nosocomial infection surveillance system,⁶ have been proposed for the diagnosis of VAP, but have also been criticised for non-specificity.⁷ The addition of quantitative culture results from endotracheal secretions, or more complex samples including protected specimens, brushings or bronchoalveolar lavage fluid (BAL),³ to the clinical scoring systems for diagnosing VAP goes some way to reducing the potential overdiagnosis of VAP.⁸ Quantitative microbiological cultures are limited by their reduced sensitivity in patients who have been treated with antimicrobials in the preceding 3 days.^{9–10} Finally, laboratories do not routinely test for atypical pathogens. Recent work has highlighted a significant prevalence of anaerobic bacteria,¹¹ viruses¹² and fungi^{13–14} in intubated patients, although their role in VAP remains unclear.

Ideally, therefore, in diagnosing VAP we could distinguish colonisation from infection causing pulmonary inflammation, and remain confident of a negative diagnosis in a subject already receiving antimicrobial treatment. In addition, our ideal diagnostic test would allow the safe withholding of antibiotic treatment from patients who do not have true VAP.

A number of biomarkers have therefore been evaluated for VAP. These have been extensively reviewed⁷ and, while C-reactive protein, procalcitonin, and soluble triggering receptor expressed on myeloid cells (sTREM) are promising, an ideal biomarker for VAP diagnosis remains to be identified.

The article by Conway-Morris *et al* in this edition of *Thorax* (see page 201) adds important new data in the search for a biomarker for VAP.¹⁵ The authors have already progressed the field in diagnosis of VAP in a study published earlier this year comparing bronchoscopic and non-bronchoscopic methods for diagnosis of VAP,⁸ showing a 39% reduction in reported VAP incidence by a change of practice study involving bronchoscopic- and microbiologically based methods. Impressively this was associated with a 21% reduction in antibiotic use, which just failed to reach statistical significance. In the current study, the investigators evaluate the ability of a number of BAL inflammatory cytokines to distinguish patients with or without VAP in whom the diagnosis was

clinically suspected. Using quantitative microbiological culture of BAL as their reference standard, with 10^4 cfu/ml being defined as a positive culture confirming VAP,¹⁶ of 72 patients with clinically suspected VAP only 17 (24%) had positive cultures. Samples underwent anaerobic culture in addition to the standard Health Protection Agency-recommended methods for processing and culturing BAL fluid for the diagnosis of VAP.¹⁷ Of the remaining “non-VAP” samples, 22 cultured organisms at $<10^4$ cfu/ml, while 33 were sterile. The bacteriological methods used were rigorous, with samples being processed in both the National Health Service (NHS) laboratory and the research laboratory, with high qualitative and quantitative concordance for the organisms isolated.

BAL cytokine concentrations were compared between these clinically and microbiologically defined groups of “VAP” and “non-VAP”, and a further control group of age-matched healthy volunteers. Patients with VAP had higher BAL interleukin 1 β (IL-1 β), IL-8, granulocyte colony-stimulating factor (G-CSF) and macrophage inflammatory protein-1 α (MIP-1 α) than the non-VAP ventilated group and healthy controls. Interestingly, unlike in the previous study,^{7–18} sTREM concentrations did not differ between the VAP and non-VAP groups.

The investigators further analysed these data to identify which biomarkers had best discriminatory value in confirming or refuting the microbiological diagnosis of VAP: IL-1 β and IL-8 emerged as the most promising biomarkers. In this group of patients, a BAL IL-1 β concentration <10 pg/ml was a powerful negative predictor of VAP (post-test probability of having VAP was 2.8%). Higher IL-1 β concentrations did not necessarily positively predict VAP. IL-8, in contrast, at concentrations of >4000 pg/ml strongly predicted a diagnosis of VAP, with a post-test probability of confirming VAP of 75%. (However, as the authors discuss, the patient numbers with concentrations in excess of 4000 pg/ml were small.) None of the other cytokines which were upregulated in the VAP group had similar predictive values. Combining cytokines in statistical models did not further improve the predictive value over IL-1 β or IL-8 alone. Serum cytokines, in contrast, did not differ between the VAP and non-VAP groups.

The data suggest that a low BAL IL-1 β concentration might improve confidence in withholding antibiotics in a patient with clinically suspected VAP, while

waiting for microbiological cultures, while a higher IL-8 would support the decision towards antibiotic prescription. The authors argue that while confirming a negative culture can take up to 48 h BAL cytokine results can be made available by 4 h, allowing more targeted decision making.

However, as the authors acknowledge, there are some potential limitations. To validate a novel biomarker requires confidence in the reference standard. The study uses quantitative BAL as the reference standard to confirm the presence or absence of VAP, against which the biomarkers are then validated. However, not all studies have confirmed that it is superior to clinical diagnosis alone (reviewed in Rea-Neto *et al*), nor that it gives a consistent diagnosis. However, as the authors point out, histological confirmation of pneumonia is not a practical reference for validation, and quantitative microbiological samples are increasingly accepted by the critical care community.

A further limitation of the study is the exclusion of patients who had had an antibiotic change within 72 h. The BAL cytokine data are not necessarily applicable to this group, and the discriminatory value of IL-8 and IL-1 β in such a cohort has not been addressed. In addition, more patients in the “non-VAP” group were already on antibiotic treatment at the time of bronchoscopy and BAL. It is possible that the pre-existing antimicrobial treatment may lead to a false-negative microbiological diagnosis, misclassifying patients with VAP as “non-VAP”. The authors argue that the higher IL-1 β and IL-8 responses occurred only in the true “VAP” group—that is, with cfu $>10^4$ /ml—but it remains difficult to be confident that the “non-VAP” group (with positive cultures but at lower cfu) are not in fact true patients with VAP in evolution: this study did not include follow-up of the patients in each group to give their emerging clinical course. In addition, it would be interesting to know about the use of corticosteroids or immunomodulatory antibiotics such as tetracyclines or macrolides in each of the groups as these might affect measured cytokine profiles.

The ecology of the bacteria isolated in the cohort is a little unusual in that no *Pseudomonas* species were identified in the VAP group: the applicability of the cytokine data in the absence of a highly typical intensive care unit (ICU) pathogen³ therefore needs to be considered, and reproduced in other ICU cohorts. In addition, the study did not include viral

cultures. Up to 22% of patients intubated for >48 h had evidence of virus recoverable from tracheobronchial secretions in one study.¹² Whether viruses in VAP contribute to an actual pneumonic process remains unclear.

Very interestingly, IL-1 β and IL-8 concentrations were similar in the patients with VAP with typical pathogenic microbes and those with microbes usually considered to be less pathogenic, for example coagulase-negative staphylococci or *Candida* species. It is not clear how many of these patients had simultaneous positive cultures for other microbes. If these are the sole isolates, the cytokine data suggest that these microbes do in fact induce an inflammatory response: by extrapolation, therefore, they may require treatment. Isolated *Candida* cultures, for example, are not routinely treated in clinical practice. This article raises the possibility that such "non-pathogenic" microbes are in fact true pathogens in the setting of VAP.

Finally, the use of a bronchoscopically derived biomarker for clinical practice presents certain practical considerations. BAL is labour intense, and cannot be performed safely in all ICU patients (and as in the methods described, the sicker patients with higher ventilatory support requirements were excluded). This study was carried out by one investigator across two clinical sites. Highly precise methods were used to perform BAL by the investigator, and it is unlikely that this methodological rigour could be easily reproduced by multiple clinicians in different ICUs in clinical practice. Although the authors have previously demonstrated that BAL-based microbiological techniques can improve the diagnosis of VAP⁸ there remains widespread high variability

in the use of bronchoscopic sampling across ICUs throughout Europe.¹⁹

Nevertheless this study adds significantly to the diagnosis and understanding of VAP. The utility of these biomarkers for VAP in BAL now requires further evaluation in larger multicentre studies. It will be particularly interesting to see if the application of biomarker-based decisions could safely reduce antibiotic prescription, and whether biomarker measurements help our understanding of the role of atypical or "non-pathogenic" microbes in VAP.

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Is vitamin D deficiency important in the natural history of COPD?

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Vitamin D consists of a group of fat-soluble prohormones, the most important of which are vitamin D2 and D3, with measurement of 25-hydroxyvitamin D (25-OHD) closely representing a person's vitamin D2 and D3 status. D2 (ergo-

calciferol) is plant and fungal derived, while vitamin D3 (cholecalciferol) is made from 7-dehydrocholesterol in the skin. This conversion of 7-dehydrocholesterol to previtamin D3 is governed by both the intensity and appropriate wavelength of the ultraviolet (UV) B irradiation reaching 7-dehydrocholesterol. Adequate amounts of vitamin D3 can be made in the skin after only 10–15 min of sun exposure at least twice a week without sunscreen. However, with longer exposure to UVB rays, equilibrium is achieved in the skin and the vitamin degrades as fast as it is generated. Serum concentrations of vitamin D have been found to vary with age, race, sex,