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—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 20054 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
specification were 69% and 75%, respectively, indicating the need for better diagnostic systems. More complex scoring systems combining clinical and microbiological data, for example the clinical pulmonary infection score\(^5\) 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,\(^11\) viruses\(^1\)\(^2\) and fungi\(^1\)\(^3\)\(^4\) 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 reviewed\(^7\) 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.\(^5\) 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 59% 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,\(^6\) 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 35 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 1β (IL-1β), IL-8, granulocyte colony-stimulating factor (G-CSF) and macrophage inflammatory protein-1α (MIP-1α) than the non-VAP ventilated group and healthy controls. Interestingly, unlike in the previous study,\(^7\)\(^15\) 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-1β and IL-8 emerged as the most promising biomarkers. In this group of patients, a BAL IL-1β concentration <10 pg/ml was a powerful negative predictor of VAP (post-test probability of having VAP was 2.8%). Higher IL-1β 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-1β 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-1β 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\(^7\)), 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-1β 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-1β and IL-8 responses occurred only in the true “VAP” group—that is, with cfu >10⁹/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) pathogen\(^3\) 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. 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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**REFERENCES**


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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-OH D) closely representing a person’s vitamin D2 and D3 status. D2 (ergocalciferol) 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,