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The diagnosis of ventilator-associated pneumonia using non-bronchoscopic, non-directed lung lavages

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Abstract *Objectives*: (1)To assess the diagnostic utility of quantitative cultures of non-bronchoscopic lung lavage (NBL) in ventilator-associated pneumonia and evaluate the role of the Bacterial Index; (2) To assess the predictive value of NBL surveillance quantitative cultures in ventilated patients; (3) To evaluate the Clinical Pulmonary Infection Score (CPIS) system in ventilated patients.
Design: A prospective comparison of NBL with bronchoscopic bronchoalveolar lavage and protected specimen brush.
Setting: Three intensive care units in academic tertiary care centres.
Patients: 145 adults ventilated for 72 h, with and without clinical signs of pneumonia.
Interventions: Sampling of lower airway secretions by NBL, bronchoalveolar lavage and protected specimen brush.
Main outcome measures: Diagnostic reliability of quantitative cultures, Bacterial Index and CPIS.
Results: 34 episodes of clinical ventilator-associated pneumonia were documented in 32 patients. 9 episodes were confirmed by concordant blood/pleural culture or post-mortem lung examination. Qualitative concordance of the predominant pathogen between sequential NBL: bronchoalveolar lavage and protected specimen brush was 83 %. Sensitivity and specificity of non-directed bronchial lavage at a threshold of 10^4 CFU/ml were 68 % and 70 % respectively ($p = 0.003$) and were comparable with the bronchoscopic methods. However, the low positive predictive value of NBL indicates that quantitation in the absence of clinical signs is unlikely to be useful. Bacterial Index did not improve discriminatory power of quantitation compared with bacterial load of predominant organism. Mean CPIS for confirmed pneumonia cases was 8.4 ± 1.01, significantly higher than for non-pneumonia observations ($p = < 0.0001$).
Conclusion: NBL is a simple, safe, cheap, readily-available method of diagnosing ventilator-associated pneumonia with comparable diagnostic accuracy to bronchoscopic techniques. Quantitation of respiratory tract cultures can exclude pneumonia in patients with equivocal clinical signs. The diagnostic threshold should vary depending on the length of ventilation, likelihood of pneumonia and antibiotic administration. The Bacterial Index is a flawed mathematical device that has no contributory role in pneumonia diagnosis. The CPIS has some diagnostic role in selected cohorts of ventilated patients.

Key words Ventilator-associated pneumonia · Non-bronchoscopic lung lavage · Quantitative culture · Bacterial index

Introduction

Ventilator-associated pneumonia (VAP) is the most common infection documented in intensive care [1, 2] and is associated with high mortality [3]. Accurate diagnosis of VAP is an important clinical goal, allowing treatment with targeted antibiotics.

The clinical and bacteriological diagnosis of VAP is imprecise [4, 5, 6]. Distal respiratory secretions can be obtained for culture by bronchoalveolar lavage (BAL) [7] and protected specimen bronchial brushing (PSB) [3, 8, 9]. However these techniques are expensive, have recognised morbidity [10] have produced conflicting results [11, 12, 13, 14] and may not influence outcome [15]. Non-bronchoscopic lung lavages (NBL) have demonstrated comparable diagnostic accuracy [16, 17, 18] but have not been prospectively compared with both BAL and PSB in the same patient, ante-mortem. We evaluated diagnostic utility of: (a) quantitative culture of NBL, BAL and PSB in VAP; (b) serial NBL surveillance cultures in predicting onset of pneumonia; (c) the use of the Bacterial Index (BI) [12]; and (d) Clinical Pulmonary Infection Score (CPIS).

Methods

Study population

Three intensive care units in university hospitals in Cardiff, two with a mixed medical-surgical intake and one receiving adult cardiothoracic patients, participated in the study from July 1995 to May 1997. Study patients were aged 18 years or more and had been mechanically ventilated for 72 h (but with no clinical signs of pneumonia prior to 72 h). Ethical approval was granted by the local ethics committee and informed written consent was obtained from relatives of the patients.

Clinical definitions

Criteria for clinical suspicion of VAP were modified from those of the Centres for Disease Control (CDC) [19]. Confirmed pneumonia was diagnosed by either histopathological confirmation (ante-mortem or by autopsy performed within 96 h of death), or from concurrent isolation of a VAP pathogen from distal respiratory samples and from pleural fluid or blood culture, with no other apparent source of infection. Clinical suspicion of VAP was diagnosed from chest X-rays showing new, persistent (> 24 h) and/or progressive infiltrate, abscess, cavitation or suspected empyema, and an increase in the volume and purulence of suctioned secretions and *one or more* of the following: pyrexia ≥ 38 °C for more than 4 h; blood leucocytosis ≥ 11×10^9/l; and an increase in fraction of inspired oxygen (FIO_2) of 0.2 needed to maintain arterial oxygen saturation, sustained for > 4 h. Alternative sources of sepsis were sought in all patients with suspected pneumonia. A clinical diagnosis of VAP was made by the attending clinician before proceeding to bronchoscopy, and was judged retrospectively by the response to antibiotics and the exclusion of other causes of the clinical signs. Multiple episodes of pneumonia were included if > 1 week had elapsed since clinical and partial radiological resolution of the prior episode.

VAP was considered absent if: no histological signs of pneumonia were found at autopsy within 96 h of death; chest X-ray infiltrates were absent; symptoms resolved without antibiotics within 24 h; or if a definitive alternative cause was established for any episode of fever, hypoxaemia, leucocytosis or change in nature of tracheal secretions [20]. Indeterminate observations were those where an alternative source existed for at least some of the clinical signs or the clinical response to antibiotics was equivocal or non-evaluable because of patient death.

Data collection

Data collected for each patient were: age, sex, duration of hospitalisation, duration of ventilation, mortality, Acute Physiology and Chronic Health Evaluation II score, and for each respiratory sampling: Clinical Pulmonary Infection Score (CPIS), axillary body temperature, peripheral blood leucocyte count, volume and nature of tracheobronchial secretions obtained by routine suctioning, change in FIO_2 in previous 4 h, ratio of partial pressure of oxygen in arterial blood to FIO_2, appearance of chest radiograph if performed within 24 h of sampling, use of continuous haemofiltration, and duration and type of antibiotics administered. Adverse events associated with the three sampling techniques were recorded.

Study protocol

On study entry all patients underwent sequential NBL, BAL and PSB sampling for quantitative culture between 72–96 h of commencing ventilation. Thereafter, samples were obtained from patients remaining free of clinical and radiological signs of pneumonia, (criteria defined below), twice weekly by NBL, whilst ventilated. Where clinical signs of pneumonia were found after 72 h, samples were obtained by all techniques if a bronchoscope was available, or by NBL alone.

Clinical Pulmonary Infection Score (CPIS)

This scoring system incorporates weighted clinical and radiological variables (Table 1) to indicate the likelihood that a patient has pneumonia [17, 21]. The CPIS was evaluated against clinical diagnoses made using the modified CDC criteria.

Sampling techniques

For each respiratory sampling observation, specimens were obtained by the three techniques within 10 min of each other. To limit contamination of the bronchoscope, the PSB was obtained before BAL [22]. NBL sampling was performed either first or last (random numbers design).

Non-directed bronchial lavage: the patient was pre-oxygenated for 5–10 min with 100 % oxygen and a sterile, disposable swivel adapter was inserted between the endotracheal tube and the ventilator tubing [17]. A standard 59 cm, 12 gauge (4 mm diam.) tracheal catheter (Argyle Sherwood Medical, UK) was attached to a sterile syringe filled with 20 ml sterile saline, after aseptic removal of the proximal catheter hub. The catheter was advanced down the endotracheal or tracheostomy tube until resistance was met (generally at approximately 45–50 cm). The saline was injected over 10 s and immediately reaspirated into the syringe. On study entry

Table 1 Clinical Pulmonary Infection Score (CPIS) Adapted from references 17, 21

Clinical criterion	Range	Weighted score
Temperature °C (Average in previous 12 hours)	36.0–38.0 38.0–39.0 > 39.0 or < 36.0	0 1 2
White blood cell count ($\times 10^9$/l) (from same day as respiratory sampling)	4–11 11–17 < 4 or > 17	0 1 2
Nature and average volume of aspirated endotracheal secretions in previous 24 h	Scanty Moderate/profuse but non-purulent Moderate/profuse and purulent	0 1 2
Gas exchange ratio (PaO_2/FIO_2kPa: values averaged over previous 12 h)	> 33 < 33	0 2*
Chest radiograph infiltrates**	none patchy/diffuse localised	0 1 2

* If ARDS, score = 0; ** Chest X-rays were performed on the day of clinical diagnosis (if VAP suspected) or within 24 h (if no signs of VAP) of sampling, and reported by the senior attending clinician or radiologist.

and during episodes of clinical VAP, lavages were taken by the clinical investigators, and at other times by experienced qualified nurses and physiotherapists.

Protected specimen brush: bronchoscopic techniques were performed by one of the clinical investigators. Patients were sedated with propofol or midazolam. Brushings were taken by fibreoptic bronchoscope (BF 200: Olympus KeyMed, USA). The tip of the bronchoscope was directed towards a radiographic infiltrate if present, or it was directed to the right lower lobe. After wedging the instrument at the sampling site, the 160 cm, 2.3 mm diameter brush (TeleMed Systems, USA) was inserted through the sampling port, and down the working channel of the bronchoscope until it extended a few centimetres beyond the distal end [9]. The polyethyleneglycol plug was discharged and the inner catheter was advanced into distal bronchoalveolar secretions, sampling by rotation of the brush. The brush was withdrawn into the protective sheath and removed from the bronchoscope. The distal 2 cm of the brush was aseptically cut into 2 ml of sterile saline.

Bronchoalveolar lavage: mini-BAL rather than standard BAL was used, due to ethical concerns over potential adverse effects caused by BAL, in a study population in whom the majority did not have a clinical indication to perform the technique [8]. The bronchoscope was directed and wedged as for PSB sampling [22]. Sterile saline (20 ml) was instilled and immediately reaspirated into a sterile syringe.

Microbiologic processing

Specimens were cultured within 45 min. of collection. Bronchoalveolar lavage and NBL specimens were homogenised. Quantitation was performed with dipstrips (Bacteruritest strips; Mast Diagnostics, UK) on chocolate agar [23]. Additionally 0.1 ml inocula of the 1:2 and 1:20 dilutions were spread on chocolate agar and centrifuged deposits of the samples were inoculated onto 5% horse blood agar, chocolate agar, and MacConkey agar. Cultures were incubated at 37 °C aerobically or in air enriched with 5% CO_2. Anaerobic culture was performed if an empyema or abscess was detected. Serial tenfold dilutions of vortexed PSB specimens were prepared to a 1:1000 dilution and 0.1 ml inocula of the original specimen and each dilution were plated onto chocolate agar [24]. In addition, 0.1 ml inocula of the undiluted sample was plated onto blood agar and MacConkey agar and incubated as above.

Colony counts were expressed as CFU/ml: 24 and as a Bacterial Index, i.e., the sum of the $\log_{10}$ CFU/ml for all the isolates cultured from a single respiratory tract specimen [12].

Bacteriological definitions

Qualitative concordance indicates that the same species were isolated from specimens taken concurrently from the same patient. Quantitative concordance indicates the level of numeric agreement of bacterial counts between specimens taken concurrently from the same patient.

Statistical analysis

The reference standards used to define sensitivity, specificity and predictive values for the sampling techniques were: (a) confirmed VAP episodes; and (b) clinically suspected VAP episodes, as defined in the Clinical definitions section above. Sensitivity was calculated as the proportion of *all* VAP episodes (confirmed and/or clinically suspected VAP) that yielded an accepted VAP pathogen at or above the threshold under evaluation. Specificity was calculated from all specimens taken from patients *without* confirmed/clinically suspected VAP at the time of sampling, that yielded an accepted VAP pathogen below the threshold under evaluation [25]. Specimens that yielded an accepted VAP pathogen at or above the threshold under evaluation, were deemed true or false positive when taken from patients with or without confirmed/clinical VAP respectively, at the time of sampling. Specimens that yielded an accepted VAP pathogen below the threshold under evaluation, were deemed true or false negative when taken from patients with or without confirmed/clinical VAP, respectively. Statistical analysis was performed with the SPSS statistical software package (SPSS Inc., USA). Quantitative concordance was assessed with Pearson's correlation coefficient (r^2) to assess concordance between techniques (r^2 values > 0.75 indicate good concordance). Optimal interpretive thresholds for quantitative data were found for sequential integer threshold values of the interpreted variable, taking that yielding the most significant p value from Fisher's exact test as the optimum threshold.

Results

One hundred and forty-five patients were recruited (Table 2.) and 367 NBL, 174 BAL and 170 PSB specimens were analysed. Thirty-four episodes of confirmed or clinically suspected VAP occurred in 32/145 patients. Four episodes were confirmed by post-mortem lung histopathology; one patient had macroscopic lung abscess and cavitation at autopsy; three patients had concurrent bacteraemia with an identical organism in NBL cultures; and one patient had a pleural empyema with an

Table 2 Characteristics of study population*

	Patients with VAP (n = 32)	Patients without VAP (n = 104)	Patients of indeterminate status (n = 9)
Age, yr	52.6 +/– 20.2	56.3 +/– 17.9	58.3 +/– 13.2
Sex, M/F	19/13	51/53	5/4
Length of hospitalisation pre-ventilation, d	4.2 +/– 6.9	8.0 +/– 17	8 +/– 7.0
Total duration of ventilation, d	16.1 +/– 13.6	10.7 +/– 7.8	13.9 +/– 9.6
APACHE II on admission	19.4 +/– 6.4	22.9 +/– 9.6	23.7 +/– 8.3
ICU mortality, n (%)	18 (56)	48 (47)	8 (80)
Obstructive lung disease, n (%)	0 (0)	8 (7.8)	1 (11)
Continuous haemofiltration, n (%)	10 (31)	33 (32)	7 (78)
Immunosuppressed, n (%)	1 (3.1)	8 (7.8)	2 (20)
Admitting condition, n (%)			
Cardiac failure	3 (9.3)	11 (10.7)	1 (11.1)
Neurologic disease	3 (9.3)	8 (7.8)	0 (0)
Trauma	6 (18.8)	14 (13.5)	0 (0)
Post cardiac surgery	0 (0)	9 (8.7)	2 (22.2)
Other post-surgical	11 (34.4)	32 (31)	4 (44.4)
Miscellaneous	9 (28)	30 (28.4)	2 (22.2)

Definition of abbreviations: APACHE = acute physiology and chronic health evaluation; $p > 0.05$ for all comparisons; * Values are means +/– SD

identical organism in NBL and pleural fluid cultures. The mean CPIS at clinical diagnosis for these nine confirmed episodes was 8.44 ± 1.01. Seven of the nine episodes occurred whilst the patient was undergoing continuous haemofiltration. The mean CPIS for *all* VAP episodes (n = 34) was 7.6 ± 1.4 compared to 4.1 ± 1.8 found for the 200 observations where pneumonia was clinically absent (according to modified CDC criteria) or was excluded at postmortem and a CPIS could be calculated ($p < 0.0001$). For *all* VAP episodes (n = 34), at the optimum CPIS threshold of 7, sensitivity was 85%, specificity 91%, positive predictive value 61%, and negative predictive value 96%.

Post-mortem lung histopathology was performed in five patients who had shown no clinical evidence of pneumonia. Two underwent complete bilateral lung dissection confirming absence of pneumonia. Three others showed no evidence of pneumonia in a total of 4 to 8 bilateral, superficial and centrally-located lung samples. The causes of death diagnosed by post-mortem histology in these five cases were pulmonary oedema (n = 1), peritonitis (n = 2), cerebral abscess (n = 1), cerebral trauma (n = 1).

Microbiological results

The range of organisms isolated during clinical episodes of pneumonia is shown in Table 3. Quantitative concordance (r^2) between $\log_{10}$ counts was 0.73 for the NBL and BAL comparison, 0.7 for NBL and PSB, and 0.84 for BAL and PSB.

Among the 28 pneumonia episodes for which all three specimen types were taken, the predominant VAP pathogen was qualitatively concordant in 24 episodes (83%). Taking significant thresholds of 10^4 CFU/ml for NBL and BAL, and 10^3 CFU/ml for PSB specimens, 69% of paired NBLs and BALs, 73% of paired NBLs and PSBs and 64% of paired BAL and PSBs, yielded the same predominant VAP pathogen.

Ten observations in 9 patients were classified as indeterminate with a mean CPIS of 5.6 ± 1.8. In 7/10 of these observations the predominant organism load for NBL was $< 10^4$ CFU/ml. The bacteriological data from indeterminate observations was excluded from the analysis.

Diagnostic parameters for the three sampling techniques, using count of the predominant accepted VAP pathogen, are summarised in Table 4. Group "All patients" depicts diagnostic reliability for all sampling episodes/observations (excluding 10 indeterminate observations in 9 patients), irrespective of antibiotic administration (i.e., n = 34 sampling observations for VAP episodes and n = 323 sampling observations for non-VAP observations in 136 patients). Group B is a subgroup of the total study population and depicts diagnostic reliability of specimens taken from the cohort of patients (n = 84) who were antibiotic-free for at least 48 h before sampling, or in whom the predominant VAP pathogen isolated was resistant to the antibiotics being administered (total of 183 NBL, 77 BAL and PSB specimens). The reliability of NBL was broadly comparable with bronchoscopic techniques for all patients and group B.

Table 3 Results from clinical VAP episodes

Patient no.	CPIS	Isolated organisms	NBL (CFU/ml)	BAL (CFU/ml)	PSB** (CFU/ml)	A/b
1. #	6	P. aeruginosa Proteus sp. Acinetobacter sp.	10^{10} 10^6 10^6	*	*	Cxm + Met; 3d
2.	6	Enterobacter sp.	10^4	10^2	10^3	Caz + Met; 3d
3.	8	Staph. aureus CNS MRSA E. coli	10^4 10^2 10^2	 10^4	NG	Flu + Rif; 2d
4.	7	H. influenzae M. catarrhalis	10^6 10^6	10^8 10^8	10^4 10^4	Nil
4.	8	Staph. aureus Acinetobacter sp. P. aeruginosa	10^4 10^2 10^2	10^4 10^2 10^2	10^3 10^4	Imi + Ery; 1d
5.	7	Staph. aureus H. influenzae	10^6 10^6	10^5 10^6	10^3 10^4	Coa + Gen; 1d
6.	7	Acinetobacter sp. Viridans streptococci	NG	10^4 10^5	10^4 10^4	Nil
7.	7	Pseudomonas sp. MRSA	NG	10^7	10^2 10^3	Cxm + Met; 3d
8.	8	MRSA	10^4	*	*	SDD; 12d
9.	7	P. aeruginosa	10^6	*	*	Nil
10. #	9	Acinetobacter sp.	10^7	10^6	10^3	Cip; 2d
11. #	8	Staph. aureus	10^7	*	*	SDD; 16d
12.	9	Acinetobacter sp.	10^8	10^9	10^5	Vanc; 2d
13.	8	Acinetobacter sp.	10^8	10^9	10^5	Vanc + Rif; 3d
14.	6	Acinetobacter sp.	10^5	10^8	10^4	Nil
14.	8	Acinetobacter sp. Proteus sp.	10^8 10^8	10^4	10^7 10^5	Nil
15. #	8	MRSA Acinetobacter sp.	10^6 10^8	10^7 10^7	10^6 10^6	Vanc; 1d
16.	9	MRSA P. aeruginosa	10^5 10^2	10^5 10^4	10^4 10^1	Amp.; 3d
17. #	10	Enterobacter cloacae	10^2	10^4	10^3	Nil
18.	7	P. aeruginosa	10^6	10^8	10^1	Nil
19.	8	H. influenzae S. pneumoniae Viridans streptococcus	10^2 10^2	10^2 10^4 10^4	10^1 10^1 10^2	Nil
20.	7	H. influenzae P. aeruginosa E. coli	10^7 10^4 10^5	*	*	Nil
21.	6	Klebsiella sp.	10^8	10^7	10^6	Nil
22. #	8	MRSA Acinetobacter sp.	10^2 10^1	10^5 10^4	10^4 10^1	Pen + Ctx; 5d
23. #	10	Sterile	NG	NG	NG	Ctx + Ery; 1d
24.	8	E. coli	10^4	10^4	10^2	Flu; 2d
25.	6	E. coli	10^5	10^2	*	Gen; 2nd
26.	9	Sterile	NG	NG	NG	Nil
27.	9	Stenotro. maltophilia	10^2	10^4	10^3	Nil
28.	8	H. influenzae MRSA	10^6 10^3	10^7 10^4	10^5 10^3	Nil

Table 3 continued

Patient no.	CPIS	Isolated organisms	NBL (CFU/ml)	BAL (CFU/ml)	PSB** (CFU/ml)	A/b
29.	9	Acinetobacter sp.	10^8	10^4	10^1	Nil
30. #	8	E. coli	NG	NG	10^1	Flu + Rif; 5d
31. #	8	MRSA	10^9	10^9	10^6	Vanc + Rif; 1d
32.	9	MRSA	10^4	10^2	10^2	Vanc + Clip; 1d

* = specimen not obtained; ** = CFU/ml of PSB diluent
Definition of abbreviations: CFU/ml = colony forming units per ml; A/b = antibiotics being administered; d = days of antibiotics prior to sampling; # = confirmed case of VAP; Staph. aureus = Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus; P. aeruginosa = Pseudomonas aeruginosa; CNS = coagulase-negative staphylococci; H. influenzae = Haemophilus influenzae; M. catarrhalis = Moraxella catarrhalis; E. coli = Escherichia coli; Stenotro. maltophilia = Stenotrophomonas maltophilia; Flu = flucloxacillin; Vanc = vancomycin; Rif = rifampicin; Gen = gentamicin; Amp = ampicillin; Cip = ciprofloxacin; Imi = imipenem; Ery = erythromycin; Ctx = cefotaxime; Caz = ceftazidime; Cxm = cefuroxime; Met = metronidazole; Pen = Benzylpenicillin; Coa = coamoxiclav; SDD = selective decontamination of digestive tract.
Note. Patient no. 23 had indistinguishable S. aureus isolates cultured from blood and respiratory secretions 24 h prior to study entry.

Table 4 Diagnostic reliability parameters for respiratory sampling techniques using count of predominant accepted VAP pathogen

	Group					
	All patients			B		
	NBL	BAL	PSB #	NBL	BAL	PSB #
Threshold (CFU/ml)*	10^4	10^4	10^3	10^4	10^4	10^3
Sensitivity (%)	74	76	68	72	89	68
Specificity (%)	70	71	86	40	48	64
PPV (%)	17	35	54	14	33	50
NPV (%)	96	93	95	90	92	85

* Threshold of predominant accepted VAP pathogen; #, CFU/ml of predominant accepted VAP pathogen in PSB diluent; NBL = non-bronchoscopic lung lavage; BAL = bronchoalveolar lavage; PSB = protected specimen brush; PPV = positive predictive value; NPV = negative predictive value. Definition of Groups; All patients = All sampling observations irrespective of antibiotic administration ($n = 34$ sampling observations for VAP episodes and $n = 323$ sampling observations for non-VAP observations in 136 patients); Group B: Observations in the antibiotic-free or antibiotic-resistant cohort ($n = 26$ sampling observations for VAP episodes and $n = 157$ sampling observations for non-VAP observations in 84 patients). For All patients and Group B, sensitivity was calculated as the proportion of all VAP episodes (confirmed and/or clinically suspected) that yielded an accepted VAP pathogen = or > threshold under evaluation and specificity was calculated from specimens taken from patients without confirmed VAP and/or clinical suspicion of pneumonia at the time of sampling. Analysis excludes bacteriological data from 10 indeterminate observations taken from 9 patients.

No improvement in reliability was found using the Bacterial Index (BI) as the diagnostic criterion compared with the predominant organism load. Statistical analysis defined the optimal interpretive threshold values for BI as: 6 for NBL specimens, ($p = 0.004$); 8 for BAL, ($p = 0.003$); and 10 for PSB ($p = 0.00001$). The diagnostic reliability parameters for the three techniques at a BI of 5 (a threshold proposed from previous studies [21, 32]) are shown in Table 5.

Eighteen episodes of pneumonia occurred in patients receiving antibiotics at the onset of clinical signs; in 8 of these the predominant organism, although sensitive to the administered antibiotics, was isolated at > 10^4 CFU/ml. Fourteen of 34 episodes (41%) yielded significant polymicrobial cultures in at least one of the specimen types. In 7/14 episodes, the load of other organisms was within 2 logarithms of that for the predominant organism.

Mean serial NBL bacterial loads for patients without VAP are shown in Fig. 1. Ninety-two NBLs were taken during non-pneumonia observations in which antibiotics had not been given for at least 48 h. Fifty-nine (64%) of these yielded a VAP pathogen at > 10^4 CFU/ml. Of these, 43 (73%) were taken at > 10 days of ventilation, but twelve (13%) were taken at study entry. In all 12 cases, either PSB or BAL yielded the same pathogen at similar counts.

Twenty-two pneumonia episodes occurred after 72 h of ventilation (Table 6.). In 9 of these episodes, the count of the predominant organism had risen, compared to specimens taken up to 72 h before diagnosis of clinical pneumonia. In the remaining 13 episodes, the pre-pneumonia specimen was taken > 72 h before diagnosis or antibiotics had been introduced in the interval. Pre-pneumonia surveillance cultures yielded the implicated

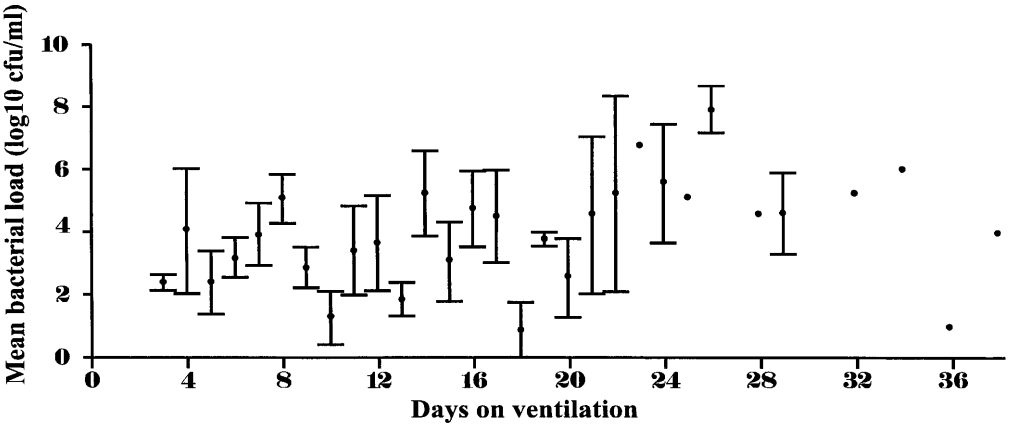

Fig. 1 Mean serial NBL bacterial loads for patients without VAP. *Capped bars* indicate one standard error of the mean (SEM). *Points without SEM bars* indicate sampling observations on less than two patients

pathogen in 14/22 episodes of pneumonia. Only 4 VAP episodes yielded a predominant organism differing from that isolated from the preceding NBL.

Antibiotics were selected on the basis of surveillance cultures and modified according to subsequent cultures from the three sampling techniques. Sixteen of 32 patients died whilst ventilated, of whom 9/16 had clinical signs of VAP at death with 8/9 receiving appropriate antibiotics.

The average recovered volume of fluid with NBL was 4 ml. Three episodes of transient hypoxaemia were noted during NBL. Four patients developed minor bronchial haemorrhage immediately after PSB. No adverse events were associated with BAL.

Discussion

This study highlights problems in the interpretation of quantitative cultures in diagnosis of VAP. VAP occurs along a bacteriologic continuum [26] and a fixed threshold for quantitative culture ignores many factors that influence the change from colonisation to infection [27]. This threshold should reflect the duration of mechanical ventilation as well as the pre-test probability of pneumonia [28] the influence of antibiotics, the pathogenic potential of isolates and immune status of the patient [2]. Many surveillance lavages (64 %) taken from patients without pneumonia and in the absence of antibiotics, yielded a VAP pathogen at $> 10^4$ CFU/ml, mostly at > 10 days mechanical ventilation. This increase in bacterial load colonising the lung with duration of ventilation is well documented [29] and affects the diagnostic value of quantitative culture if a fixed threshold is adopted [4, 30]. For specimens taken at 7 days or more of mechanical ventilation, raising the diagnostic threshold from 10^4 to 10^6 CFU/ml increased specificity markedly from 40 % to 73 % with little change in sensitivity.

In 11/34 episodes, antibiotics were introduced less than 72 h before diagnosis of clinical pneumonia, mak-

Table 5 Diagnostic reliability parameters for respiratory sampling techniques using Bacterial Index

	Group					
	All patients			B		
	NBL	BAL	PSB	NBL	BAL	PSB
BI Threshold = 5						
Sensitivity (%)	62	68	84	58	80	85
Specificity (%)	53	65	72	46	42	46
PPV (%)	14	28	36	15	32	36
NPV (%)	94	91	96	87	86	96

NBL = non-bronchoscopic lung lavage; BAL = bronchoalveolar lavage; PSB = protected specimen brush; PPV = positive predictive value; NPV = negative predictive value. Definition of Groups: All patients = All specimens irrespective of antibiotic administration ($n = 34$ sampling observations for VAP episodes and $n = 323$ sampling observations for non-VAP observations); Group B: Specimens taken when patient was antibiotic-free or receiving antibiotics ineffective against all the isolated organisms from the same specimen ($n = 25$ sampling observations for VAP episodes and $n = 117$ sampling observations for non-VAP observations in 68 patients). For All patients and group B, sensitivity was calculated as the proportion of all VAP episodes (confirmed and/or clinically suspected) that yielded a BI = or > the threshold under evaluation and specificity was calculated from specimens taken from patients without confirmed VAP/clinical suspicion of pneumonia at the time of sampling. Analysis excludes bacteriological data from 10 indeterminate observations taken from 9 patients.

Table 6 Serial NBL quantitative culture results relative to day of onset of VAP, for the 22 episodes of pneumonia occurring after 72 h of ventilation

Patient no.	Day*	NBL quantitative culture (CFU/ml)	A/b
1.	– 10	Proteus 10^4	Imi; 5d
	– 6	Proteus 10^4, Candida spp. 10^2	Imi; 9d
	– 3	Proteus 10^5, E. coli 10^7	Nil
	0	P. aeruginosa 10^{10}, Proteus 10^6	Cxm + Met; 3d
4.	– 6	Moraxella catarrhalis 10^5	Nil
	– 2	M. catarrhalis 10^4, H. influenzae 10^3	Nil
	0	M. catarrhalis 10^6, H. influenzae 10^6	Nil
	+ 5	Acinetobacter 10^5	Cip; 5d
4.	– 10	Acinetobacter 10^4, M. catarrhalis 10^5	Nil
	– 8	M. catarrhalis 10^7, Staph. aureus 10^4	Nil
	– 3	No growth	Nil
	0	Staph. aureus 10^4, Acinetobacter 10^2, P. aeruginosa 10^2	Imi. + Ery.; 1d
	+ 4	No growth	Imi; 5d
	+ 7	Serratia spp. 10^4, P. aeruginosa 10^2, viridans streptococci 10^5	Nil
5.	– 3	H. influenzae 10^5	Nil
	0	Staph. aureus 10^6, H. influenzae 10^6	Coa. + Gen. 1d
	+ 4	Staph. aureus 10^6, Acinetobacter 10^6, H. influenzae 10^6	Coa; 5d
8.	– 4	No growth	Cxm + met; 3d
	0	MRSA 10^4	SDD; 12d
	+ 3	Pseudomonas aeruginosa 10^2	Vanc.; 3d
9.	– 7	No growth	Cxm + met; 3d
	– 3	No growth	Cxm + met; 7d
	0	P. aeruginosa 10^6	Nil
	+ 6	Klebsiella 10^2	Vanc. + cip; 6d
10.	– 5	No growth	Nil
	0	Acinetobacter 10^7	Cip; 2d
	+ 6	Acinetobacter 10^2	Imi; 6d
11.	– 4	Acinetobacter 10^1	SDD; 11d
	0	Staph. aureus 10^7	SDD; 15d
14.	– 4	Acinetobacter 10^1, Candida 10^4	Nil
	0	Acinetobacter 10^5	Nil
	+ 3	Candida 10^2	Imi; 3d
14.	– 3	Proteus 10^8, Acinetobacter 10^3	Nil
	0	Acinetobacter 10^8, Proteus 10^8	Nil
	+ 5	Proteus 10^4, P. aeruginosa 10^4	Imi; 5d
17.	– 2	P. aeruginosa 10^2	Nil
	0	Enterobacter cloacae 10^2	Nil
	+ 5	No growth	Imi + gen; 5d
18.	– 5	No growth	Pen. + Ctx; 3d
	– 1	P. aeruginosa 10^5	Pen; 7d
	0	P. aeruginosa 10^6	Nil
20.	– 3	H. influenzae 10^5	Erythromycin; 2d
	0	H. influenzae 10^7, P. aeruginosa 10^4, E. coli 10^5	Nil
	+ 4	P. aeruginosa 10^2	Cip; 4d
22.	– 2	No growth	Pen. + Ctx; 3d
	0	MRSA 10^2, Acinetobacter 10^1	Pen. + Ctx; 5d
24.	– 4	E. coli 10^6, Staph. aureus 10^5, CNS 10^2	Nil
	0	E. coli 10^4	Flucloxacillin; 2d
25.	– 4	E. coli 10^4	Nil
	– 1	E. coli 10^4	Gen; 1d
	0	E. coli 10^5	Gen; 2d
	+ 2	No growth	Gen; 4d
	+ 6	MRSA 104	Nil
27.	– 2	Stenotro. maltophilia 10^5	Coa + cip; 3d
	0	Stenotro. maltophilia 10^2	Nil
	+ 7	Stenotro. maltophilia 10^2	Flu + gen; 7d

For contination of Table 6 please see the next page

Table 6 continued

Patient no.	Day*	NBL quantitative culture (CFU/ml)	A/b
28.	– 6	MRSA 10^7	Cxm; 3d
	– 1	H. influenzae 10^8, MRSA 10^5	Nil
	0	H. influenzae 10^6, MRSA 10^3	Nil
	+ 3	Acinetobacter 10^1	Vanc + cip; 3d
29.	– 3	No growth	Nil
	– 1	Acinetobacter 10^8	Nil
	0	Acinetobacter 10^8	Nil
	+ 3	Acinetobacter 10^5	Cip; 3d
30.	– 3	E. coli 10^2, Candida sp. 10^5	Flu + rif; 2d
	0	No growth	Flu + rif; 5d
31.	– 3	MRSA 10^6	Nil
	– 1	MRSA 10^9	Nil
	0	MRSA 10^9	Vanc + rif; 1d
32.	– 6	No growth	Cxm. + met; 3d
	– 2	MRSA 10^2	Nil
	0	MRSA 10^4	Vanc + cip; 1d

Definition of abbreviations: CFU/ml = colony forming units per ml; A/b = antibiotics being administered; d = days of antibiotics prior to sampling; Day* = days relative to onset of VAP; Staph. aureus = Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus; P. aeruginosa = Pseudomonas aeruginosa; CNS = coagulase-negative staphylococci; H. influenzae = Haemophilus influenzae; M. catarrhalis = Moraxella catarrhalis; E. coli = Escherichia coli; Stenotro. maltophilia = Stenotrophomonas maltophilia; Flu = flucloxacillin; Vanc = vancomycin; Rif = rifamipicin; Gen = gentamicin; Cip = ciprofloxacin; Imi = imipenem; Ery = erythromycin; Ctx = cefotaxime; Cxm = cefuroxime; Met = metronidazole; Pen = Benzylpenicillin; Coa = coamoxiclav; SDD = selective decontamination of digestive tract.

ing interpretation of quantitative cultures difficult [31, 32]. For the cohort of patients that were receiving ineffective or no antibiotics at the time of sampling, specificity was only 40 % compared to 70 % for all observations (threshold = 10^4 CFU/ml of predominant VAP pathogen), with little difference in sensitivity (72 % compared to 74 %; Table 4). This may be because ineffective antibiotic therapy encourages overgrowth in the lower airways [29, 32].

Interpretation of results is limited by lack of an accepted diagnostic reference standard for all VAP episodes [26, 32]. However, the diagnostic reliability of quantitative NBL was comparable with bronchoscopic techniques (Table 4.) and good qualitative concordance was found between bronchoscopic and NBL cultures, consistent with previous findings [8, 16, 17, 18, 33]. Lower positive predictive values (PPV) were found for NBL, reflecting a bias in sampling. The additional NBL surveillance specimens tended to show progressively higher counts with duration of ventilation. This weighted the comparison of PPV, with many observations showing high counts in NBLs in the absence of pneumonia. If histologically confirmed pneumonia and/or PSB growth of $\geq 10^3$ CFU/ml are used as the reference standards (as in previous studies [11, 13]), (n = 19 episodes), the sensitivity of NBL at a threshold of 10^4 CFU/ml was 75 %.

Bronchoscopic techniques allow collection of lung tissue for histology and culture as well as alveolar fluid for rapid microscopic detection of intracellular organisms [34]. These investigations are not possible using NBL. Mini-BAL (20 ml) collects insufficient alveolar cells for microscopic examination [22].

Using the Bacterial Index (BI) did not improve discriminatory power of quantitative cultures (Table 5.). BI assumes that every species present in the lung contributes synergistically to the disease process, irrespective of known pathogenic potential. Two studies in ventilated patients have concluded that BI improves the discriminatory power of quantitative cultures [21, 35] although other workers disagree [8, 36]. Incorporating organisms of negligible significance such as coagulase-negative staphylococci or yeasts into Bacterial Index calculations [12, 21] would have profound implications for antimicrobial usage. The relative contribution of each isolate in a polymicrobial culture to the disease process in VAP is unknown.

Quantitation is unnecessary for routine NBL surveillance cultures in patients with no clinical signs of pneumonia, because it has low positive predictive value in this context. Rising bacterial counts were found in many sequential specimen sets (Table 6.), but the fluctuations in bacterial burden observed, meant the clinical value of this *quantitative* data was limited, as opposed to the conclusion of previous studies [17, 37]. *Qualitative* surveillance findings, in combination with clinical signs, were crucial in deciding early, appropriate antibiotic therapy for patients who developed VAP [17].

The CPIS identifies patients with a high likelihood of VAP [37]. It was 7 or more in 29/34 pneumonia epi-

sodes, 8 or more in 8/9 confirmed episodes and 6 or less for the 5 patients where pneumonia was excluded at autopsy. It was thus more sensitive than BAL/PSB, although the number of patients with confirmed VAP was small. The major confounding factor affecting CPIS is the extensive use of continuous haemofiltration in intensive care, which misleadingly lowers body temperature [38].

The clinical value and accuracy of quantitative bronchoscopic culture in comparison with qualitative/quantitative tracheal aspirate culture has been questioned [32, 33]. One potential role of quantitative NBL culture is in eliminating a diagnosis of VAP in patients with equivocal clinical signs, because of its high negative predictive value. Quantitation allows evaluation of relative loads in polymicrobial cultures and thus may influence extent of antibiotic coverage. Quantitative culture should be performed only when clinical suspicion of pneumonia rises, instituting antibiotic therapy guided by prior surveillance cultures. The diagnostic threshold for quantitative culture should be based on the count of the predominant accepted VAP pathogen. This should vary with the duration of ventilation, effects of current antibiotic therapy, nature of the isolates and level of clinical suspicion.

NBL is a safe, inexpensive procedure that can be performed rapidly by medical, nursing and physiotherapy staff. Our results suggest that it may be useful as a first-line technique for patients with clinical suspicion of VAP, as it has comparable diagnostic accuracy with bronchoscopic techniques [8, 16, 17, 18] and is much more convenient to perform. Twice weekly qualitative surveillance NBLs can predict the causative organism of subsequent VAP episodes in most cases [17, 37] provided that new antibiotics are not introduced in the interval between surveillance sampling and NBLs taken at onset of VAP. Further studies of NBL, evaluating larger numbers of patients with histologically confirmed VAP are required to clearly define its role in surveillance and diagnosis of this illness.

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