Resolution of Infectious Parameters after Antimicrobial Therapy in Patients with Ventilator-associated Pneumonia

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Although recommended durations of antimicrobial therapy for ventilator-associated pneumonia (VAP) range from 7 to 21 d, these are not based on prospective studies and little is known about the resolution of symptoms after start of antibiotics. Resolution of these symptoms was investigated in 27 patients. VAP was diagnosed on clinical, radiographic, and microbiological criteria, including quantitative cultures of bronchoalveolar lavage. All patients received appropriate antibiotic therapy. Highest temperatures, leukocyte counts, $Pa_{O_2}/F_{I_{O_2}}$ ratios, and semiquantitative cultures of endotracheal aspirates were recorded from start of therapy until Day 14. Resolution was defined as the first day that these parameters fulfilled the following definition: temperature $\leq 38^{\circ}$ C, leukocytes $\leq 10 \times 10^9$/L, $Pa_{O_2}/F_{I_{O_2}}$ ratio ≥ 25 kPa, and no or +1 of bacterial growth of etiologic pathogens in cultures of endotracheal aspirate. VAP was caused by Enterobacteriaceae (n = 14), *P. aeruginosa* (n = 7), *S. aureus* (n = 6), *H. influenzae* (n = 3), and *S. pneumoniae* (n = 1). *H. influenzae* and *S. pneumoniae* were eradicated from tracheal aspirates, whereas Enterobacteriaceae, *S. aureus*, and *P. aeruginosa* persisted, despite *in vitro* susceptibility to antibiotics administered. Significant improvements were observed for all clinical parameters, most apparently within the first 6 d after start of antibiotics. Newly acquired colonization, especially with *P. aeruginosa* and Enterobacteriaceae, occurred in the second week of therapy. Six patients developed a recurrent episode of VAP, four of them with *P. aeruginosa*. Clinical responses to therapy for VAP occur within the first 6 d of therapy, endotracheal colonization with Gram-negative bacteria persists despite susceptibility to therapy, and acquired colonization usually occurs in the second week of therapy and frequently precedes a recurrent episode.

Ventilator-associated pneumonia (VAP) is the most frequent intensive care unit (ICU)-acquired infection among patients receiving mechanical ventilation (1). VAP has been associated with an attributable mortality of approximately 30% depending on the pathogen isolated (2, 3), especially when initial antibiotic therapy is inappropriate (4). Of all antibiotics prescribed for therapy in intensive care patients, approximately 50% are administered for respiratory tract infections (5). As a result, VAP has an important impact on patient morbidity and mortality, as well as on the costs for health care.

Diagnosing VAP is problematic because of the difficulties in distinguishing colonization of the respiratory tract from infection of the lung parenchyma, and the absence of a clinically useful gold standard (6). In most ICUs the diagnosis is based on a combination of clinical, radiographic, and microbiological criteria. Although the sensitivity of these criteria is high, specificity is low and antibiotics are frequently prescribed unnecessarily. The enormous use of antibiotics creates a constant threat for selection and induction of resistant pathogens and exposes patients to adverse effects. Therefore, reducing antibiotic use may have several beneficial aspects. Reductions in antibiotic use may be achieved by preventing the development of infections, optimizing the accuracy of diagnostic procedures (7), or reducing the length of treatment.

Little is known about the optimal duration of antibiotic therapy for VAP. According to guidelines from the American Thoracic Society, VAP due to *Haemophilus influenzae* and methicillin-sensitive *Staphylococcus aureus* should be treated for 7 to 10 d, whereas episodes caused by *Pseudomonas aeruginosa* and *Acinetobacter* spp. should be treated for at least 14 to 21 d (8). However, these recommendations are not based on the results of prospective studies. Furthermore, there is sparse information about resolution of infectious parameters associated with VAP after institution of appropriate antimicrobial therapy.

Garrard and A'Court described a gradual normalization of a combination of clinical, microbiological, and radiographic parameters after the institution of antibiotic therapy (9). And Montravers and coworkers demonstrated, with a second bronchoscopy 3 d after institution of antimicrobial therapy, that appropriate therapy results in a rapid bacteriological clearance of the distal airways. However, the effects on clinical parameters were less evident (10). The aims of the present study were to describe the clinical and microbiological response to appropriate antimicrobial therapy in patients with VAP.

METHODS

Setting

The study was conducted in the ICU of the University Hospital Maastricht from January 1, 1998 until January 1, 1999. The ICU is a 16-bed ward harboring a mixed population of medical, surgical, trauma, and neurological patients.

Patients and Data Collection

All patients who received mechanical ventilation for > 48 h and met the criteria for VAP (Table 1) were enrolled. Patients received empiric antibiotic treatment according to culture results of previously obtained endotracheal aspirates, and antibiotics were streamlined upon definite culture results from bronchoalveolar lavage (BAL). Empiric therapy was considered appropriate when pathogens isolated in significant amounts from BAL samples were, *in vitro*, susceptible to the antibiotics. Routine surveillance included microbiological analysis of endotracheal aspirates on admission and subsequently twice weekly. The duration of antibiotic treatment was determined at the discretion of treating physicians. Demographic data (i.e., age, sex, preexistent diseases, and length of hospital stay before admission) and APACHE II admission scores were assessed as described by Knaus and coworkers (11). Clinical, laboratory, and microbiological variables were collected daily during 14 d starting at Day 0. The following data were obtained: highest temperature, arterial oxygen tension (Pa_{O_2})/fractional inspired oxygen ($F_{I_{O_2}}$) ratio, leukocytes in the peripheral blood, and semiquantitative cultures of the endotracheal aspirate. In case of a

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clinical suspicion of VAP, endotracheal aspirates were obtained just before bronchoscopy.

Microbiological Analysis

Samples of endotracheal aspirates were inoculated on blood agar (Becton Dickinson, Columbia agar), cysteine lactose electrolyte-deficient agar (Becton Dickinson, CLED 43331), *Haemophilus* selective agar, and *Streptococcus* and *Staphylococcus* selective agar. The number of colony-forming units (cfu) was determined semiquantitatively by the four quadrant method and classified as follows: no growth, $+1 = 10^2$ cfu/ml, $2+ = 10^3$ cfu/ml, $3+ = 10^4$ cfu/ml, and $4+ = 10^6$ cfu/ml (10). We previously demonstrated that there is a good correlation between semiquantitative and quantitative analyses of endotracheal aspirates. All semiquantitative cultures with 4+ growth had quantitative culture results of $\geq 10^6$ cfu/ml (12, 13).

Resolution of Infectious Parameters

Four parameters (i.e., highest temperature, leukocyte count in peripheral blood, Pa_{O_2}/FI_{O_2} ratio, and semiquantitative culture result of endotracheal aspirate) were used to determine the resolution of infectious parameters. We adopted cut-off points for "normality" based on common clinical practice: highest temperature $\leq 38°$ C, leukocyte count in peripheral blood $\leq 10 \times 10^3/mm^3$, Pa_{O_2}/FI_{O_2} ratio ≥ 25 kPa, and no or +1 of bacterial growth, of bacteria similar to those isolated from BAL samples, in semiquantitative cultures of endotracheal aspirates. Resolution of ventilator-associated pneumonia was defined as the first day after start of therapy at which all parameters were "normal."

For additional analyses patients were grouped according to the pathogens causing VAP: community-acquired pathogens like *Streptococcus pneumoniae*, *H. influenzae*, and *S. aureus* (group A); Enterobacteriaceae (group B); and *P. aeruginosa* (group C).

Statistical Analysis

Kaplan-Meier analyses were used to depict the changes of infectious parameters in time. Cox-regression analysis was used to determine the prognostic value of changes of individual infectious parameters. Daily changes of these parameters per patient were analyzed with linear regression and differences were tested with the one-sample *t* test. Mann–Whitney test (MW) and Kruskal–Wallis (KW) tests were used to compare groups. A p value < 0.05 was considered significant.

RESULTS

Twenty-seven patients (16 male) fulfilled the diagnostic criteria for VAP (Table 2). Antibiotic agents used before and after bronchoscopy, microorganisms isolated from cultures from endotracheal aspirates and BAL samples, results of the chest radiographs, duration of antibiotic treatment for VAP, time to extubation, length of ICU stay, and resolution of fever from individual patients are listed in Table E1 (*see* online data supplement—Table E1). Mean duration of antibiotic treatment for VAP was 11.5 ± 2.9 d (median 13 d, range 7–14 d). VAP was accompanied by bacteremia in four patients (15%). Most cases of VAP were caused by Enterobacteriaceae and *P. aeruginosa*, accounting for 67% of causative microorganisms; in 14 patients VAP was caused by Enterobacteriaceae and in 7 patients by *P. aeruginosa.* In all, 35 bacterial species were isolated in significant amounts from BAL fluid. Seven episodes were polymicrobial (*see* online data supplement—Table E1). Initial antimicrobial therapy was appropriate in all cases. Despite *in vitro* susceptibility of isolated pathogens to the prescribed antimicrobial therapy, infection persisted in one patient (patient 1). On suspicion of superinfection a second BAL was performed after 6 d of treatment, which, again, yielded *P. mirabilis* with susceptibility for the initial administered antimicrobial therapy. These antibiotics were continued and the patient fully recovered.

A second episode of VAP was diagnosed in 6 patients (22%). In three patients a new pathogen (in all cases *P. aeruginosa*) was isolated, which was resistant to the antibiotics administered during the first episode. These patients (7, 8, and 9) acquired tracheal colonization with *P. aeruginosa* 5, 9, and 10 d after start of antimicrobial therapy, respectively, and developed infection 4 (7 and 8) and 21 d after discontinuation of therapy. In the other three patients (16, 25, and 27) similar bacterial species as those isolated in the first episode were associated with the second episode of VAP (i.e., *P. aeruginosa*, *Escherichia coli*, and *S. aureus*). Only *E. coli* causing a new episode of VAP (patient 16), 8 d after discontinuation of therapy, was resistant to the antibiotics used during the first episode. Patient 25 acquired new colonization with *S. aureus* 4 d after a 14-d course of therapy with flucloxacillin and a recurrent episode of VAP was diagnosed 2 d later. Finally, patient 27 received 14 d of treatment with meropenem for *P. aeruginosa* VAP. During this period, tracheal colonization persisted and pulmonary infiltrates persisted. When fever recurred 6 d after discontinuation of antibiotics, a second BAL was performed and a recurrent episode of VAP was diagnosed.

Mean durations of antibiotic treatment were comparable for patients with VAP caused or not caused by *P. aeruginosa*; 12.7 ± 1.7 d (median 12.5, range 7–14 d) versus 11.1 ± 3.1 d (median 14, range 10–14 d), respectively (p = 0.4, MW), and between those who did or did not experience a second episode of VAP,

TABLE 1. CRITERIA USED FOR THE DIAGNOSIS OF VENTILATOR-ASSOCIATED PNEUMONIA*

Criteria	
A	More than three positive of the following four:
	1. Rectal temperature above 38.0° C or below 35.5° C
	2. Blood leukocytosis ($> 10 \times 10^3/mm^3$) and/or left shift or blood leukopenia ($< 3 \times 10^3/mm^3$)
	3. > 10 leukocytes per high-power field in Gram stain of tracheal aspirate
	4. Positive culture from tracheal aspirate and
B	New, persistent, or progressive infiltrate on chest radiograph and
C	More than one positive of the following three:
	1. Positive quantitative culture of a sample obtained by BAL (cut-off point $> 10^4$ cfu/ml)
	2. Positive blood culture unrelated to another source and obtained within 48 h before and after respiratory sampling
	3. Positive pleural fluid culture in the absence of previous pleural instrumentation

Definition of abbreviation: BAL = bronchoalveolar lavage.
* Pneumonia was diagnosed if A and B and C were positive.

TABLE 2. BASELINE CHARACTERISTICS OF THE STUDY PATIENTS

Characteristic	Number of Patients ($n = 27$)
Male/female	16/11
Mean age, yr (range)	62.6 (34–79)
APACHE II score, mean ± SD	21.8 ± 7.4
Days in hospital prior to ICU, median (range)	11 (0–37)
Days in ICU, median (range)	34 (10–78)
Time to extubation in days after start of antibiotic treatment of VAP, median (range)	17 (5–65)
Antibiotic use on admission, number of patients (%)*	21 (76)
Underlying diseases, number of patients (%)	
Cardiovascular disease	11 (41)
Respiratory disease	7 (26)
Neurological disease	5 (19)
Gastrointestinal disease	4 (15)
Neoplastic disease	4 (15)
Diabetes mellitus	4 (15)
Alcoholism or drug abuse	2 (7)
Renal insufficiency	2 (7)
Immunodeficiency	1 (4)

Definition of abbreviations: ICU = intensive care unit; VAP = ventilator-associated pneumonia.
* Prophylaxis not included.

10.3 ± 3.1 d (median 10, range 7–14 d) versus 11.5 ± 2.8 d, respectively (median 13, range 10–14 days) (p = 0.3, MW).

Seven patients died during hospitalization, and three of them died in the ICU. In these three patients, VAP was diagnosed 17–59 d before death. In the other four patients, VAP was diagnosed 28–49 d before death. Therefore, in none of these patients did death seem to be directly attributable to VAP.

Resolution of Infectious Parameters

After institution of antibiotic therapy a gradual fall in the mean number of colony-forming units (cfu) in cultures of endotracheal aspirates was observed (Figure 1A). The bacterial load dropped from 5.4 cfu/ml on Day 0 to 1.4 cfu/ml on Day 15. However, there were extreme differences when comparing individual microorganisms. There was persistent colonization with *P. aeruginosa* in all patients, whereas bacterial colonization with *S. aureus*, *H. influenzae*, and *S. pneumoniae* completely disappeared from endotracheal aspirates (Figure 2A). Tracheal colonization with Enterobacteriaceae persisted in almost half of the patients. Interestingly, acquired tracheal colonization was demonstrated in several patients, usually during the second week of antimicrobial therapy and most frequently with *P. aeruginosa* (Figure 2B).

Time to Resolution

All four parameters of infection improved significantly with time (Figure 1A–1D). On Day 0, averages of the four variables were leukocyte count, 14.2 ± 5.4 × 10^3/mm^3, which decreased 0.15 × 10^3/mm^3/d ($p < 0.01$); highest temperature, 38.8 ± 0.5° C, which decreased 0.05° C/d ($p < 0.01$); Pa_{O_2}/FI_{O_2}, 26.3 ± 10.5 kPa, which increased 0.8 kPa/d ($p < 0.01$); and the bacterial load in endotracheal aspirates, 5.4 ± 1.2 cfu/ml, which decreased 0.2 cfu/ml/d ($p < 0.01$). No significant differences were found when comparing the daily changes in leukocyte counts, highest temperatures, and Pa_{O_2}/FI_{O_2} ratios between the three groups of causative pathogens of VAP (A, B, and C), not even in the first 6 d of treatment when changes were most prominent. The changes in leukocyte counts, highest temperatures, and Pa_{O_2}/FI_{O_2} ratios were −0.36 × 10^3/mm^3, −0.1° C, and +1.2 kPa, respectively, in group A; −0.30 × 10^3/mm^3, −0.16° C, and +1.4 kPa, respectively, in group B; and −0.27 × 10^3/mm^3, −0.15° C, and +1.2 kPa, respectively, in group C (KW, NS).

For patients with abnormalities on Day 0 in temperature (> 38° C, n = 27), Pa_{O_2}/FI_{O_2} ratio(< 25 kPa, n = 17), leukocyte count (> 10^3/mm^3, n = 21), and number of cfu/ml (> 10^2, n = 27), the mean (median) duration to resolution of these parameters was 5 (3), 6 (2), 8 (6), and 10 (7) d, respectively (Figure 3). The mean (median) duration until complete resolution (i.e., for all parameters) was 9 (8) d. When excluding microbiological eradication, the mean (median) duration of resolution for the remaining three clinical parameters was 6 (6) d. Mean duration of antibiotic treatment in this group of patients was 10 ± 2.8 d (median 10, range 7–14 days), as compared with 13.1 ± 2.1 d

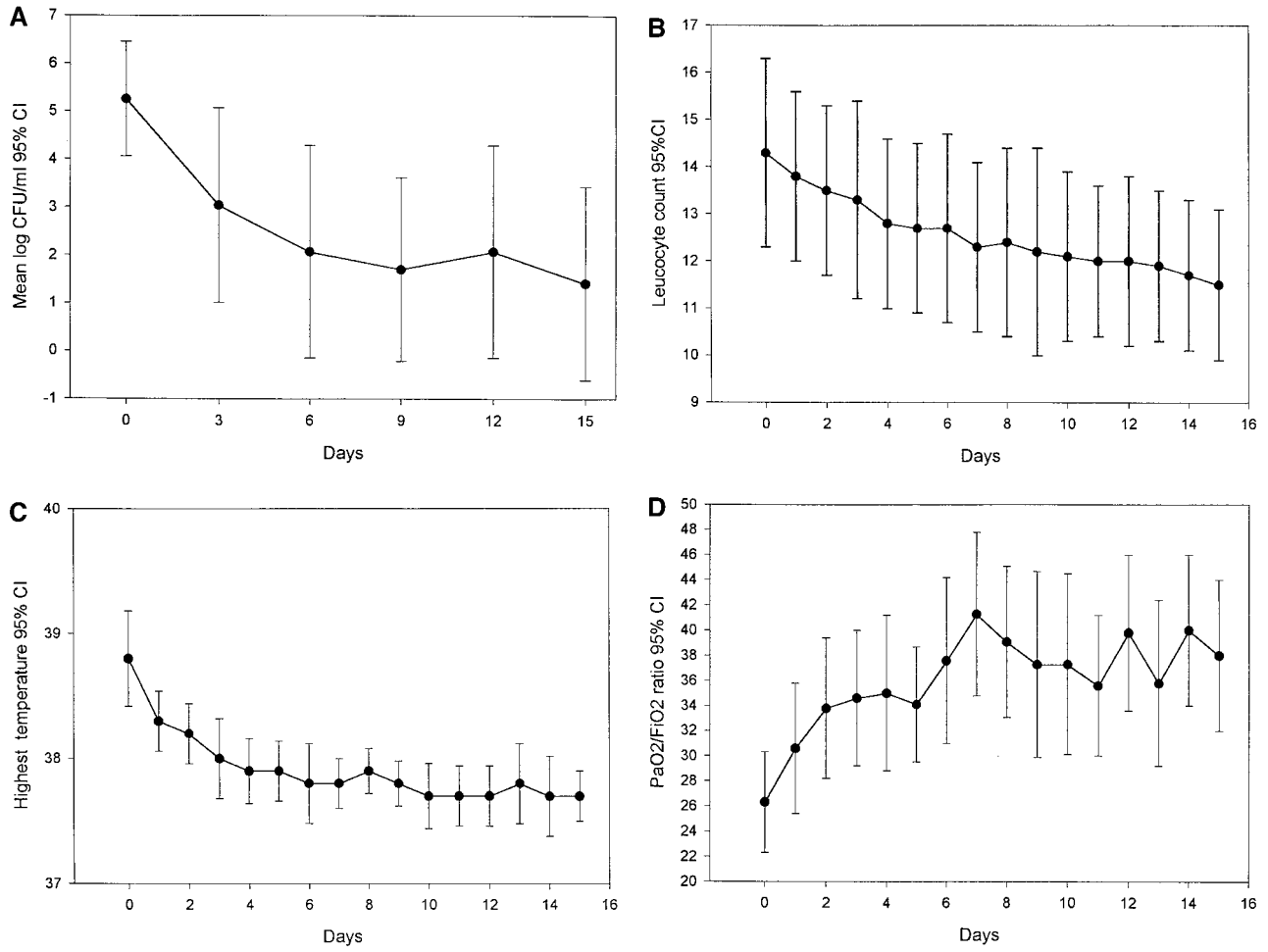

Figure 1. (*A*) Mean log colony-forming units per milliliter of endotracheal aspirate of those bacteria associated with VAP after initiation of antimicrobial treatment. (*B*) Leukocyte count after initiation of antibiotic treatment. (*C*) Highest temperature after initiation of antibiotic treatment. (*D*) Pa_{O_2}/FI_{O_2} ratio after initiation of antibiotic treatment.

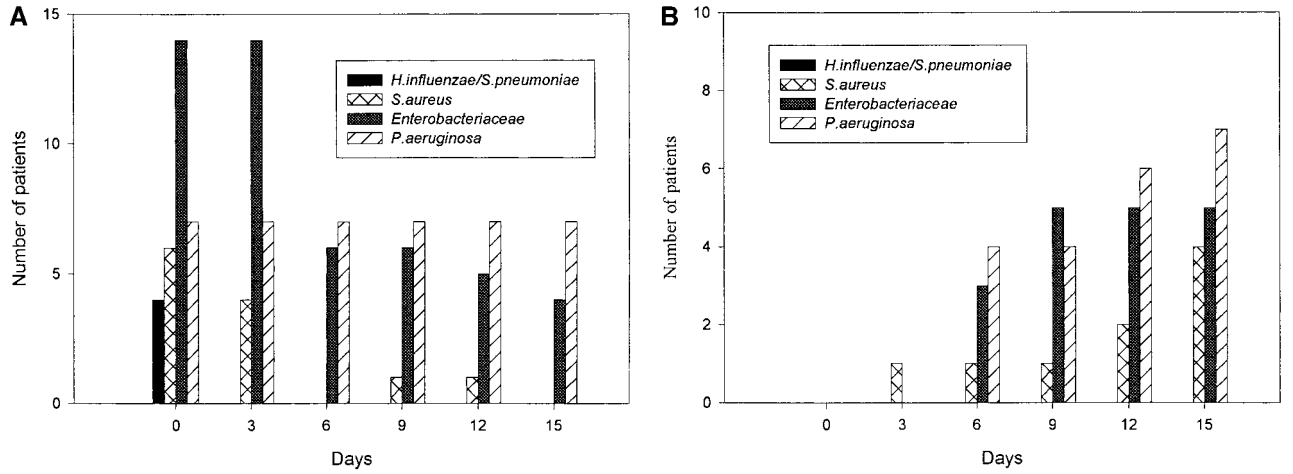

Figure 2. (*A*) Number of patients with initially isolated microorganisms from endotracheal aspirates collected in time after initiation of antibiotic treatment. (*B*) Number of patients with newly isolated microorganisms from endotracheal aspirates in time after initiation of antibiotic treatment.

(median 14, range 7–14 d) if no complete resolution occurred ($p < 0.01$, MW).The Pa_{O_2}/FI_{O_2} ratio was the only significant covariate of resolution ($p \leq 0.01$). The differences between the durations until resolution between groups A, B, C and between survivors and nonsurvivors were comparable (data not shown).

DISCUSSION

We described the resolution of infectious parameters after initiation of appropriate antimicrobial therapy for VAP. In general, resolution of clinical parameters is slow, most evident during the first 6 d of therapy and most apparent for the Pa_{O_2}/FI_{O_2} ratio. Appropriate antimicrobial therapy rapidly eradicates endotracheal colonization with *S. pneumoniae*, *H. influenzae*, and *S. aureus*, but fails to eradicate colonization with Enterobacteriaceae and *P. aeruginosa*, indicating that follow-up of this parameter is an unreliable parameter for therapy success when these pathogens are involved. Moreover, tracheal colonization with resistant pathogens frequently occurs during the second week of therapy. Based on our findings, a shorter duration of antimicrobial therapy for VAP may be as effective and may reduce the risks of colonization and subsequent infection with more resistant pathogens.

There is sparse information about the clinical and microbiological response to appropriate antimicrobial therapy in patients with VAP. This may be related to the existing problems in diagnosing VAP. In clinical practice, VAP is usually diagnosed on a combination of clinical and radiographic criteria in combination with semiquantitative cultures from tracheal aspirates. The combination of these criteria has a high sensitivity for VAP, but specificity is low (7, 14). As a result, many patients will be incorrectly diagnosed as having VAP, and a description of resolution of clinical parameters in such a population may be influenced by patients having colonization instead of infection of the respiratory tract. In the present study, quantitative cultures from BAL were added to the combination of diagnostic criteria for diagnosing VAP. Although we realize that these methods cannot be considered a gold standard, they do represent the best clinically available tools to diagnose VAP. Moreover, a recent study demonstrated that the use of invasive techniques, as used in this study, was associated with a better patient outcome and considerable reductions in antibiotic use (7). Although we found that temperature, Pa_{O_2}/FI_{O_2} ratio, and leukocyte counts improved significantly in time after initiation of antibiotic treatment, the resolution of these parameters was generally slow. The mean time to resolution of these clinical parameters was 6 d. These patients were treated for a shorter duration, suggesting that clinicians take these clinical parameters into account in deciding whether to continue or discontinue therapy.

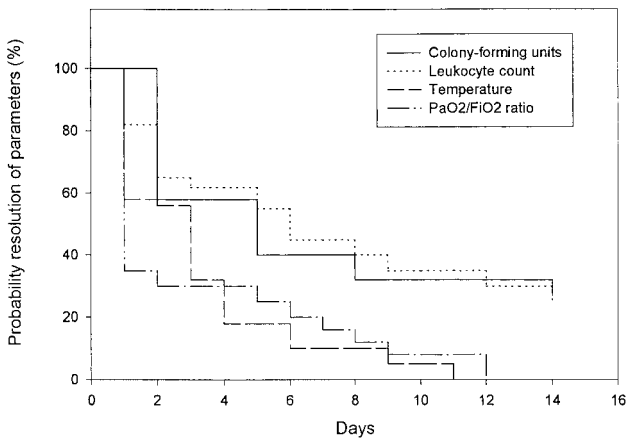

Figure 3. Probability of resolution of individual parameters after initiation of antibiotic treatment using Kaplan–Meier analysis.

In a previous study Garrard and A'Court determined the clinical response to antimicrobial therapy in 83 patients with VAP (9). The clinical response was measured by daily values of the Clinical Pulmonary Infection Score (CPIS), previously defined by Pugin and coworkers (15). The CPIS is a combination of clinical, radiographic, and microbiological criteria, with a maximum score of 12. Values of 7 or higher are considered diagnostic for VAP. Although quantitative cultures from bronchoscopic techniques are not included in the CPIS, these techniques were used for validation of the CPIS (15). Similar to our findings, they found a gradual improvement of the CPIS during the first 9 d after commencement of antibiotic therapy (9). In another study, Montravers and coworkers observed a significant decrease in temperature and increase in Pa_{O_2}/FI_{O_2} ratio within 3 d of antibiotic treatment, which was accompanied by eradication of bacteria from distal airways in the majority of patients, as demonstrated by repeated protective specimen brushes (PSB) (10).

Although interpretations of chest radiographs are important diagnostic criteria for VAP, we decided not to use this as a parameter of infection resolution. Chest radiographs are generally considered to be of limited value for defining clinical

improvement in patients with pneumonia (16–18), although rapidly deteriorating abnormalities are suggestive of either progressive or recurrent episodes of VAP.

Our findings of persistent endotracheal colonization are completely different from the reported effects of antimicrobial therapy on repeated quantitative cultures from PSB, as reported by Montravers and coworkers (10). Using cultures from PSB, they demonstrated that bacteria were eradicated in distal airways in 51 of 76 patients with VAP within 3 d after start of antimicrobial treatment (10). These findings again emphasize that endotracheal colonization is not equivalent to infection of distal airways and that bacterial eradication from endotracheal aspirates is a poor marker for determination of clinical response of VAP, especially when caused by Gram-negative bacteria. Only when VAP is caused by *H. influenzae* or *S. pneumoniae* may follow-up of endotracheal aspirates be useful.

A recurrent episode of VAP was diagnosed in six patients. The clinical response to therapy for their first episode was not different as compared with the responses observed in patients who did not develop a recurrent episode. Four of the six pathogens associated with the recurrent episodes were resistant to antibiotics used to treat the first episode, and four episodes were caused by *P. aeruginosa.* A recurrent episode of VAP, caused by *P. aeruginosa*, may result from persistent colonization, acquired colonization from exogenous sources, or selection of endogenous colonization. Rello and coworkers recently described, using molecular biotyping, that recurrent episodes of VAP caused by *P. aeruginosa* were frequently caused by peristence of the causative strains in the respiratory tract (19). Our findings of persistent endotracheal colonization of *P. aeruginosa* in all patients with *P. aeruginosa* VAP, despite appropriate antimicrobial therapy, fully support these findings. Moreover, several other patients acquired tracheal colonization with *P. aeruginosa* during therapy. Although molecular biotyping was not performed in the present study, previous analyses in our ward demonstrated that colonization and infection with *P. aeruginosa* were only sporadically caused by cross-transmission, and that selection of endogenous strains, usually colonizing the intestinal tract, was a more important route of colonization (20).

Can our findings be extrapolated to ventilated patients in other settings? The etiology of bacteria causing VAP in our ward is characterized by the absence of important resistance problems, and, because of regular surveillance of tracheal aspirates, empirical therapy was in all cases appropriate. If empirical therapy would be inappropriate, as described in other studies (2, 4), clinical responses may be completely different or even absent. The lack of a standardized approach for antibiotic prescription and discontinuation may be a possible weakness of the present study. However, the practice, as described here, is the current practice in our ICU, and was, therefore, most appropriate to investigate. Moreover, our patient population was small and causes of VAP were heterogeneous, thereby limiting extrapolation to other patient groups.

In conclusion, the clinical response to antimicrobial therapy for VAP, diagnosed with bronchoscopic techniques, occurs within the first 6 d of therapy; colonization with Gram-negative bacteria will persist in many patients; and acquired colonization, predominantly with resistant pathogens, occurs mainly in the second week of therapy. Therefore, it can be hypothesized that a duration of therapy for VAP of 7 d would be sufficient and could prevent recurrent colonization and infection with more resistant bacteria, such as *P. aeruginosa.* Currently, a duration of therapy of 14 to 21 d is recommended for VAP caused by Enterobacteriaceae or *P. aeruginosa* (8).

A strategy to treat VAP for 7 instead of 14–21 d should be tested in a prospective randomized study design. A shorter duration of therapy would reduce the selective pressure for colonization, and, thereby, the risks of recurrent infection with resistant pathogens. Moreover, a reduction in antibiotic use may reduce the risks of adverse drug events and help to control health care costs, in which costs for antibiotics are elementary.

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