Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*

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**Scientific Knowledge on the Subject:** In experimental inoculation pneumonia, nebulization of antibiotics provides high lung tissue concentrations and rapid bacterial killing.  
**What This Study Adds to the field:** Nebulized ceftazidime and amikacin provide clinical cure of ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*, including
strains with decreased susceptibility to one or both antibiotics, and may prevent per-
treatment acquisition of antibiotic resistance.

This article has an online data supplement, which is accessible from this issue's table of
content online at www.atsjournals.org".

Authors' contributions

QL carried out the study and drafted the manuscript. JY, ZL, CG participated in the
study and study analysis. GA performed measurement of plasma concentrations of
antibiotics and participated in the interpretation of the results. JJR initiated the study,
participated in the design and conception of the study and helped to improve the
draft. Members of the Nebulized Antibiotics Study Group participated directly to the
study by contributing to include patients and/or participating to the redaction of the
manuscript.
ABSTRACT

Rationale: In experimental pneumonia, nebulization of antibiotics provides high lung tissue concentrations and rapid bacterial killing.

Objectives: To assess efficacy and safety of nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by Pseudomonas aeruginosa.

Methods: Forty patients with ventilator-associated pneumonia caused by Pseudomonas aeruginosa were included in a randomized comparative phase II trial. Twenty patients infected by susceptible or intermediate strains, received nebulized ceftazidime (15 mg.kg\(^{-1}\).3h\(^{-1}\)) and amikacin (25 mg.kg\(^{-1}\).day\(^{-1}\)). Seventeen patients infected by susceptible strains received intravenous ceftazidime (90 mg.kg\(^{-1}\).day\(^{-1}\), continuous administration) and amikacin (15 mg.kg\(^{-1}\).day\(^{-1}\)). In 3 patients infected by intermediate strains, amikacin was replaced by ciprofloxacin (400 mg.12 h\(^{-1}\)).

Measurements and Main Results: After 8 days of antibiotic administration, aerosol and intravenous groups were similar in terms of successful treatment (70% vs 55%), treatment failure (15% vs 30 %) and superinfection by other microorganisms (15% vs 15%). Antibiotic-induced changes in lung computed tomography aeration were not different between groups (increase in gas volume=159 ± 460 ml vs 251 ± 583 ml; decrease in tissue volume= -58 (-77 – 25) ml vs -89 (-139 – 5) ml). Acquisition of pertreatment antibiotic resistance was observed exclusively in intravenous group. In aerosol group, 4 patients infected by intermediate strains were successfully treated. Nebulization induced an obstruction of expiratory filter in 3 patients. The obstruction caused cardiac arrest in 1 patient who fully recovered after brief cardiopulmonary resuscitation.

Conclusion: Nebulization and intravenous infusion of ceftazidime and amikacin provide similar efficiency for treating ventilator-associated pneumonia caused by
*Pseudomonas aeruginosa*. Nebulization is efficient against intermediate strains and may prevent per-treatment acquisition of antibiotic resistance.

**Word:** 259

**Key words:** Aerosol, ceftazidime, amikacin, ventilator-associated pneumonia, *Pseudomonas aeruginosa*
INTRODUCTION

Ventilator-associated pneumonia (VAP) caused by *Pseudomonas aeruginosa* (*P. aeruginosa*) is a difficult-to-treat infection associated with high rate of recurrence and frequent selection of new resistance to antibiotics despite adequate initial antimicrobial therapy (1-3). Although ceftazidime is a cephalosporin whose activity is specifically directed against *P. aeruginosa*, impaired susceptibility of *P. aeruginosa* to ceftazidime is on constant rise (4, 5). Emergence of antibiotic resistance of isolates of *P. aeruginosa* is associated with a longer duration of hospitalisation (6).

Nebulization of antibiotics offers the possibility of delivering high lung tissue concentrations of antibiotics in normal and infected lungs (7, 8). In experimental models, single daily nebulization of amikacin, a concentration-dependent antibiotic, provides a rapid and efficient bacterial killing in piglets with *Escherichia coli* inoculation pneumonia (9). In piglets with inoculation pneumonia caused by *P. aeruginosa* with reduced susceptibility, multiple daily nebulization of ceftazidime, a time-dependent antibiotic, provide greater lung deposition and more efficient bacterial killing than continuous intravenous administration (10).

Decreased susceptibility to antibiotics is a major health problem worldwide and treating infections with the lowest ecological impact on bacterial resistance, is emerging as a critical issue. Therefore, antibiotic nebulization which provides high lung tissue concentration deserves to be evaluated in patients with VAP. The present phase II study was designed to assess the efficacy and safety of combined nebulized ceftazidime and amikacin for treating VAP caused by *P. aeruginosa*. Clinical and bacteriological cure of VAP after 8 days of antimicrobial therapy was the primary endpoint. Secondary endpoints included antibiotic-induced changes of lung aeration and lung inflammation assessed by computed tomography scan and per-treatment
emergence of resistant strains. Some of the results of this study have been previously reported in the form of an abstract (11).
MATERIAL AND METHODS

Study design and patients

After informed consent was obtained from patients’ relatives, patients admitted to the Multidisciplinary Intensive Care Unit of La Pitié-Salpêtrière hospital were randomly assigned to aerosol or intravenous group in a prospective phase II trial. Eligibility criteria were: age > 18 and VAP caused by *P. aeruginosa*. VAP was defined according to clinical, biological, radiological and quantitative bacterial criteria (12). *P. aeruginosa* had to be present at concentrations ≥ 10^4 cfu.mL^{-1} in bronchoalveolar lavage (BAL) or ≥ 10^3 cfu.mL^{-1} in protected mini BAL (13). Exclusion criteria were: treatment by antibiotics active on *P. aeruginosa* > 24 hours, extrapulmonary infection, expected extubation within 3 days, severe immunosuppression, allergy to penicillins or aminoglycosides, *P. aeruginosa* resistant to ceftazidime and/or amikacin and PaO₂/FIO₂ ≤ 100 mmHg.

As previously recommended (7, 9, 10, 14), aerosol dose was determined as intravenous dose + extrapulmonary deposition in order to deliver comparable amounts of ceftazidime and amikacin in the trachea and pulmonary artery. In 17 patients, nebulization chamber, inspiratory and expiratory circuits were rinsed to assess extrapulmonary deposition (15). In the aerosol group, patients received 8 aerosols per day of 15 mg.kg^{-1} ceftazidime (120 mg.kg^{-1}.day^{-1}) for 8 days and a single daily aerosol of 25 mg.kg^{-1} amikacin for 3 days. Weaning test was authorized after 3 days of full treatment. If patients of the aerosol group were extubated before day 8, ceftazidime was continued intravenously. In the intravenous group, patients received a bolus of ceftazidime (30 mg.kg^{-1} over 30 min) followed by a continuous infusion of 90 mg.kg^{-1} day^{-1} during 8 days, and a daily amikacin bolus of 15 mg.kg^{-1}.
day$^{-1}$ over a 30-min period for 3 days. Patients infected by \textit{P aeruginosa} intermediate to ceftazidime and/or amikacin were treated in the aerosol group by nebulized ceftazidime and amikacin and in the intravenous group by ciprofloxacin.

The protocol is summarized in figure 1. Computed tomography (CT) of the whole lung was performed before initiation and after completion of antimicrobial therapy. At day 3 and 4, amikacin plasma concentrations were measured by fluorescence polarization immunoassay and ceftazidime plasma concentrations by high-performance liquid chromatography. On day 9, response to antibiotic treatment was classified by non blinded investigators in 4 categories: 1) “Cure” of VAP, defined as the association of reduction of clinical and biological signs of infection, decrease in CPIS below 6, significant lung CT reaeration, and lower respiratory tract specimens either sterile or with non-significant concentrations of \textit{P aeruginosa}. 2) Persisting VAP, defined as lack of improvement of clinical and biological signs, CPIS greater than 6, absence of CT lung reaeration with significant concentrations of \textit{P aeruginosa} persisting in lower respiratory tract specimens. 3) Recurrence of \textit{P aeruginosa} VAP, defined as initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of \textit{P aeruginosa} VAP. 4) Superinfection, defined as initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of VAP caused by pathogens other than \textit{P aeruginosa}.

**Nebulization procedure**

Nebulization was performed with vibrating plate nebulizers using specific ventilator settings (see online data supplement). To standardize and optimize the nebulization procedure, a checklist form was completed by the nurse in charge of the
patient (table 1). All adverse events were assessed for severity and for relationship to study treatment.

**Computed Tomography Measurements**

Contiguous axial 5-mm thick CT sections of the whole lung were acquired at end-expiration at day 0 and day 9 (16). Volumes of gas and tissue and total lung volumes were computed (16, 17).

Antibiotic-induced lung reaeration following 8-day treatment was measured as the increase in gas volume in lung regions characterized by multiple and disseminated foci of pneumonia and in lung areas of confluent bronchopneumonia (see figure 2 and online data supplement) (18).

**Statistical analysis**

Data are expressed as mean ± SD or as median and 25%-75% interquartile range according to data distribution. Quantitative data were compared between groups using bilateral unpaired Student’s test or Mann-Whitney rank-sum test. Changes in clinical signs and CT parameters were compared by two-way ANOVA. The statistical significance level was fixed at 0.05.

**RESULTS**

**Patients**

Over a 36-month period, 79 patients with VAP caused by *P aeruginosa* were screened for inclusion. Thirty-three patients were initially excluded for various reasons: 10 had received antibiotics active on *P aeruginosa* for more than 24 hours, 13 had positive blood cultures, 6 were expected to be extubated within 3 days, 2 had
PaO\textsubscript{2}/FiO\textsubscript{2} < 100 and 2 had *P. aeruginosa* resistant to ceftazidime or amikacin. Finally, 46 patients were enrolled in the study. Twenty-four patients were randomly assigned to the aerosol group and 22 to the intravenous group (figure 1). Six patients were secondary excluded from the study within 1 day of treatment: 3 patients for bacteremia (2 in the aerosol group and 1 in the intravenous group), 1 patient in the aerosol group for severe hypoxemia related to rapid progression of lung injury leading to severe acute respiratory distress syndrome (ADRS) within 12 hours following inclusion (PaO\textsubscript{2}/FiO\textsubscript{2} = 75) and 2 patients for *P. aeruginosa* strain resistant to ceftazidime (1 patient in the aerosol group and 1 in the intravenous group), leaving 20 patients in each group. As shown in table 2, clinical characteristics of the patients at baseline were not statistically different between groups.

In the intravenous group, all patients received the 8-day continuous infusion of ceftazidime, 17 received the 3-day amikacin administration and 3, a 3-day ciprofloxacin administration. In the aerosol group, all patients received the 3-day nebulization of amikacin whereas only 9 patients received the 8-day nebulization of ceftazidime. Eleven patients of the aerosol group were extubated during the treatment period and 3 were reintubated within 48 hrs. They received nebulized ceftazidime for 4.9 ± 1.2 days and intravenous ceftazidime for 2.9 ± 1.5 days. Four patients were initially infected by *P. aeruginosa* strains intermediate to ceftazidime and/or amikacin.

**Ceftazidime and amikacin extrapulmonary deposition**

Of the initial amount of ceftazidime inserted into the nebulizer, 5 ± 3% was retained in the nebulizer's chamber, 24 ± 10% in the inspiratory limb of the respiratory circuit and 8 ± 8% in the expiratory filter. The total extrapulmonary
deposition was 37 ± 11%. The resulting fraction of ceftazidime reaching the respiratory tract was 63% of the initial dose placed in the nebulizer chamber (120 mg.kg^{-1}), representing a daily dose of 76 mg.kg^{-1} delivered to the respiratory tract.

Of the initial amount of amikacin inserted into the nebulizer, 5 ± 5% was retained in the nebulizer's chamber, 25 ± 12% in the inspiratory limb of the respiratory circuit and 7 ± 3% in the expiratory filter. The total extrapulmonary deposition was 37 ± 13%. The resulting fraction of amikacin reaching the respiratory tract was 63 % of the initial dose placed in the nebulizer chamber (25 mg.kg^{-1}), representing a daily dose 15.7 mg.kg^{-1} delivered to the respiratory tract.

**Antibiotic treatment efficacy**

At the end of the treatment (day 9), cure of VAP was obtained in 70% of patients in nebulized group and 55% in intravenous group (p=0.33). Treatment failure with persisting VAP caused by *P aeruginosa* requiring continuation or restart of adequate antibiotics was observed in 3 patients of the nebulized group and 6 patients in intravenous group. As shown in table 3, recurrence of VAP caused by *P aeruginosa* or other microorganisms, length of stay and duration of mechanical ventilation were not significantly different between groups.

**Microbiological response to treatment and acquisition of antibiotic resistance**

Patients treated by nebulized ceftazidime and amikacin had rapid and early reduction of bacterial growth: bacterial cultures of BAL and protected mini-BAL were negative in 16 of 17 patients on day 3 and in all patients on day 5 (table 4). *P aeruginosa* re-appeared in BAL and protected mini-BAL in 2 patients on day 7 and 5
patients on day 9. At days 9, 2 patients, considered as successfully treated, had positive BAL with bacterial concentrations of $10^2$ cfu.mL$^{-1}$; 3 patients, considered as unsuccessfully treated, had positive mini-BAL $\geq 10^3$ cfu.mL$^{-1}$. Isolated strains at day 9 were all susceptible to ceftazidime and amikacin. In 4 patients initially infected by *P. aeruginosa* intermediate to ceftazidime and/or amikacin, these strains were successfully eradicated by nebulization. Pneumonia persisted in 3 patients, all infected by susceptible *P. aeruginosa*.

Patients treated by intravenous ceftazidime and amikacin had partial and delayed reduction of bacterial growth: bacterial cultures of BAL and protected mini-BAL were positive to *P. aeruginosa* in 40% of patients on days 3 and 5, and in 25% of patients on days 7 and 9 (table 4). At day 9, bacterial cultures of mini-BAL and BAL were respectively, $\geq 10^3$ cfu.mL$^{-1}$ and $\geq 10^4$ cfu.mL$^{-1}$, in 6 patients with treatment failure. Interestingly, resistant strains were isolated in BAL and protected mini-BAL from day 5. On day 7, 4 of 5 isolated *P. aeruginosa* had become intermediate or resistant to either ceftazidime or amikacin. At day 9, pneumonia persisted in 6 patients, 3 of them being infected by *P. aeruginosa* intermediate or resistant to either ceftazidime and/or amikacin.

**Changes of lung aeration and inflammation following antimicrobial therapy**

At baseline, aerosol and intravenous groups did not differ in terms of total gas volume [1082 (805-1561) ml vs 1419 (1216-1737) ml, $p = 0.17$], gas volume in regions of confluent pneumonia [86 (54-161) ml vs 64 (49-124) ml, $p = 0.41$], total tissue volume (1025 ± 269 ml vs 969 ± 242 ml, $p = 0.54$), and tissue volume in regions of confluent pneumonia (507 ± 214 ml vs 423 ± 166 ml, $p = 0.26$).
In patients of both groups successfully treated by antibiotics, gas volume increased significantly in regions with non confluent foci of pneumonia and in regions of confluent pneumonia (figure 3A). In the aerosol group, lung reaeration was observed not only in patients with VAP caused by susceptible \textit{P. aeruginosa} (figure 4) but also in patients with VAP caused by intermediate \textit{P. aeruginosa} (figure 5). Tissue volume remained unchanged in regions with non confluent foci of pneumonia and significantly decreased in regions of confluent bronchopneumonia (figure 3B). These changes were similar in both groups.

In patients in whom antimicrobial therapy failed, gas volume significantly decreased in regions with non confluent foci of pneumonia (figure 3C, left panel) and remained unchanged in regions of confluent pneumonia (figure 3C, right panel). These changes were similar in both groups.

**Adverse events**

Bronchospasm was not observed. Mean PaCO$_2$ (39 ± 4 mmHg versus 40 ± 7 mmHg, $p = 0.52$) and median PaO$_2$ [242 (194 – 256) versus 240 (199 – 259) mmHg, $p = 0.35$] were not different before and after nebulization. However, in 3 patients with initial PaO$_2$/FiO$_2$ ratio less than 200, PaO$_2$ decreased by 25% at the end of the nebulization period. One patient was excluded at the early phase of the study for severe hypoxemia related to nebulization-induced alveolar derecruitment.

Three adverse events related to obstruction of expiratory filter were reported, among which, 1 was considered as a serious adverse event. Obstruction of the expiratory filter was detected as an increase in peak airway pressure in 2 patients and sudden cardiac arrest in 1 patient. Expiratory obstruction resolved after
immediate replacement of expiratory filter. Following early cardiopulmonary resuscitation, the patient had full recovery and left the ICU at day 24.

Ceftazidime and amikacin plasma concentrations

*Plasma concentrations of ceftazidime and amikacin in the intravenous group.* As shown in table 5, following 4-day continuous intravenous administration of ceftazidime, trough concentrations were greater than 8 fold MIC of susceptible strains. Following 3-day intravenous administration of amikacin, peak plasma concentrations were greater than 5 fold MIC of susceptible strains, whereas trough concentrations were < 5 mg.L⁻¹.

*Systemic diffusion of ceftazidime and amikacin in the nebulization group.* As shown in table 5, following 4-day nebulization of ceftazidime, peak and trough concentrations were significantly lower than after intravenous administration. Following 3 daily nebulization of amikacin, peak plasma concentrations were 5 fold less than after intravenous administration, whereas trough concentrations were not different.
DISCUSSION

This clinical pilot phase II trial shows that nebulization and intravenous infusion of ceftazidime and amikacin have similar efficiency in terms of clinical and radiological cure of VAP caused by susceptible \textit{P aeruginosa}. Nebulization of ceftazidime and amikacin is also efficient to treat patients with VAP caused by intermediate \textit{P aeruginosa}. Interestingly, nebulization of ceftazidime and amikacin provides more rapid bacterial eradication from distal pulmonary samples than intravenous administration. Incidence of antimicrobial therapy failure and VAP recurrence was similar in both groups. In patients treated by aerosols, however, \textit{P aeruginosa} new growth or persistence was caused exclusively by susceptible strains, whereas in the intravenous group, 50% of \textit{P aeruginosa} strains isolated on day 9 had become intermediate or resistant to one or both antibiotics. Confirming experimental studies, nebulization did not reduce trough amikacin concentrations which are main determinants of systemic toxicity (9). The technique of nebulization is quite demanding and requires standardized aerosol procedures to optimize distal lung deposition. It can be associated with obstruction of expiratory filter and deterioration of arterial oxygenation. Such adverse effects are detected by an increase in peak airway pressure and a decrease in arterial oxygen saturation.

Efficiency of nebulized ceftazidime and amikacin for treating VAP caused by \textit{P aeruginosa}

Based on promising experimental studies (9, 10), the present clinical pilot phase II study was designed in order to assess clinical efficiency and safety of combined nebulized ceftazidime and amikacin in \textit{P aeruginosa} VAP. Cure of VAP
was observed in 70% of patients treated by nebulized ceftazidime and amikacin. Half of failures of antimicrobial therapy were caused by superinfection by other microorganisms and half by lack of eradication of *P. aeruginosa*. As a consequence, nebulization of ceftazidime and amikacin could eradicate 85% of strains of *P. aeruginosa* from the deep lung. This result is slightly superior to the one obtained with intravenous antibiotics (70%). In addition, nebulization has 2 potential advantages over intravenous administration.

First, it provides efficient cure of VAP caused by *P. aeruginosa* intermediate to amikacin and/or ceftazidime. As unanimously admitted and experimentally demonstrated (19), such a result cannot be achieved by intravenous administration. As a consequence, patients of the intravenous group infected by *P. aeruginosa* intermediate to amikacin were treated by ciprofloxacin, a molecule well known to increase the risk of antibiotic resistance in intensive care units (20, 21). Further studies are required to confirm the superiority of nebulized antibiotics for treating VAP caused by pathogens with reduced susceptibility.

Second, persisting and recurrent infection was caused by *P. aeruginosa* remaining susceptible to amikacin and ceftazidime in all patients of the nebulized group and in half of patients of the intravenous group. These findings are in accordance with a recent study showing that nebulized antibiotic combined with intravenous antibiotics decrease bacterial resistance in patients with ventilator-associated tracheobronchitis (22). In the present study, tissue concentrations far above mutant prevention concentrations (23, 24) likely reached infected lung regions following aerosols, thereby avoiding the selection of resistant strains. In the intravenous group, ceftazidime plasma concentrations were eightfold higher than MIC of susceptible *P. aeruginosa*. Trough concentrations of time-dependant
antibiotics are close to lung interstitial tissue concentrations, the critical concentrations required for killing susceptible microorganisms. Therefore, the intravenous route was as efficient as nebulization for eradicating susceptible strains infecting the lung. Since ceftazidime trough concentrations were only one to threefold higher than MIC of intermediate strains of *Pseudomonas aeruginosa*, it justifies *a posteriori*, the replacement of ceftazidime and amikacin by ciprofloxacin (25).

It is widely accepted that duration of antimicrobial therapy for VAP can be restricted to 8 days (12, 26). There is, however, some evidence suggesting that *P. aeruginosa* VAP should be treated for 2 weeks with a combination of appropriate antibiotics (27, 28). The longer duration of treatment is aimed at reducing the high incidence of *P. aeruginosa* VAP relapse, recently attributed to the presence of the type III secretion system that interferes with neutrophil functions and impairs bactericidal activity-induced antibiotics (29). In the present study, the duration of treatment was fixed at 8 days with the hope that high antibiotics lung tissue deposition and rapid bactericidal activity at the site of infection (7, 9, 10, 14) would reduce the incidence of relapse and recurrence in the aerosol group. Unfortunately, these expected benefits were not observed, suggesting that either high antibiotic concentrations do not inhibit type III secretion system or reservoirs of *P. aeruginosa*, such as oropharynx, endotracheal cuff (30) or the biofilm (31) present on the internal walls of endotracheal tube, were not affected by nebulized antibiotics (32).

**Methodological limitations**

With the small sample size of this pilot study, the power was insufficient to test a significant difference of cure rates between both study groups. A non-significant 15% absolute increase of cure of *P. aeruginosa* VAP was found at day 9 in the
nebulization vs. the intravenous group. However, the power to detect a significant
difference at an alpha risk of 0.05 was limited to 16%. An absolute difference of
41.5% would have been required to reach statistical significance at a beta risk of 0.20.
The positive trend in cure rates of *P. aeruginosa* VAP at day 9 emphasizes the need
for a further trial adequately powered.

The population study may not be entirely representative of the population of
patients with VAP caused by *P. aeruginosa* since some selection criteria may have
introduced a potential bias. As patients treated by antibiotics active against
*P. aeruginosa* for more than 24 hours were excluded and identification of
*P. aeruginosa* in a distal pulmonary sample was a prerequisite for inclusion, most
included patients were initially treated with inadequate antibiotics. Therefore the
selected population may have an increased morbidity and mortality related to
inappropriate initial antibiotic therapy (33-35). In other words a subpopulation of
patients with more severe respiratory prognostics may have been studied.

*P. aeruginosa* eradication was more frequently observed in the aerosol group
compared to the intravenous group. Such a result may have been overestimated by
the fact that some patients were extubated before the end of antimicrobial treatment,
a condition rendering difficult to obtain distal pulmonary samples. This hypothesis
seems however unlikely, since the same proportion of patients was extubated in the
intravenous group. A carry-over effect, inhibiting bacterial growth in bronchoalveolar
lavage and not indicating true bacterial killing, cannot be formally excluded.

Last but not least, 20% of patients with *P. aeruginosa* VAP had positive blood
cultures and had to be excluded because nebulization of ceftazidime and amikacin
does not provide enough plasma concentrations to treat efficiently extrapulmonary
infections (table 5).
Safety and feasibility of nebulized antibiotics in patients with VAP

Iterative daily aerosols of time-dependent antibiotics are required to ensure efficient bactericidal activity against *P. aeruginosa* (10). In order to decrease impaction of aerosol particles on ventilator circuits and optimize distal lung deposition, specific ventilator settings are required. They are all aimed at reducing turbulences of inspiratory flow and frequently impose sedation to synchronize patients. In order to guarantee appropriate antibiotics administration, checklist form was completed by the nurse in charge of the patient before and after each aerosol (table 1). Bronchospasm was not observed. Disconnection of patient from the ventilator was repeated every 3 hours for ceftazidime nebulization. Even though aerosols were generally well tolerated in terms of arterial oxygenation, a 25% decrease in PaO\textsubscript{2} at the end of aerosol was observed in 3 patients. One patient had to be excluded because of severe hypoxemia related to rapid progression of lung injury leading to severe ARDS. In this situation, disconnection from the ventilator required before each nebulization for changing the expiratory filter was considered as a risky procedure. Three adverse events related to obstruction of expiratory filter by aerosol particles were reported. One of them induced a brief cardiac arrest, outlining the need for close monitoring of airway pressure during nebulization. In order to prevent expiratory obstruction, a new expiratory filter should be inserted before each new nebulization. In order to detect arterial oxygenation impairment and progressive obstruction of the expiratory filter during the nebulization procedure, oxygen saturation and peak airway pressure alarms should be appropriately set before commencing and checklist form (table 1) should be completed after aerosol to ensure that expiratory filter is removed.
In conclusion, nebulization and intravenous infusion of ceftazidime and amikacin provide similar efficiency in terms of clinical cure of VAP caused by susceptible *P. aeruginosa*. Nebulization provides clinical cure of VAP caused by *P. aeruginosa* with reduced susceptibility and may prevent the per-treatment emergence of resistant strains. It does not reduce the incidence of VAP recurrence. These benefits are obtained only if conditions of nebulization are optimized and adverse events such as obstruction of the expiratory filter are detected early enough. A large multicenter randomized trial is required to determine whether these potential benefits outweigh the risks of serious adverse events in patients with *P. aeruginosa* ventilator-associated pneumonia.
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FIGURES LEGENDS

Figure 1 Summary of the protocol. Aerosol = Patients treated by nebulized ceftazidime and amikacin. Intravenous = Patients treated by intravenous ceftazidime and amikacin or ciprofloxacin; CT = Computed tomography; BAL = Bronchoalveolar lavage; Strain = *Pseudomonas aeruginosa* intermediate to ceftazidime (4 mg.L$^{-1}$ < minimal inhibitory concentration ≤ 32 mg.L$^{-1}$) and/or amikacin (8 mg.L$^{-1}$ < minimal inhibitory concentration ≤ 16 mg.L$^{-1}$).

Figure 2 Computed tomography assessment of lung reaeration in a patient treated by nebulization of ceftazidime and amikacin. Image 1 shows a CT section at day 0 (before antibiotic administration) at the carina level with lung regions characterized by multiple and disseminated non confluent foci of pneumonia (short arrows) and lung regions characterized by confluent pneumonia (long arrow). Image 2 shows a color encoding analysis applied to the delineated right lung parenchyma. Normally aerated lung parenchyma appears in dark grey, interstitial pneumonia and/or edema in light grey, and foci of pneumonia, either disseminated or confluent, in red. Image 3 shows the manual delineation of lung regions characterized by multiple and disseminated non confluent foci of pneumonia and those characterized by confluent pneumonia (continuous and dashed lines, respectively. Image 4 shows the corresponding CT section (according to pulmonary vessels, bronchi and mediastinal structures) at day 9, after nebulization of ceftazidime and amikacin for 8 days. Image 5 shows the color encoding analysis applied to the delineated right lung parenchyma. Image 6 shows the transposition of the delineation of both regions of interest performed at day 0, on
the CT section obtained at day 9. By referring to anatomical landmarks such as pulmonary vessels, fissures, and segmental bronchi, the limit between the region characterized by multiple and disseminated foci of pneumonia and the region characterized by confluent pneumonia was manually redrawn on the CT section obtained at day 9 according to the previous delineation performed at day 0. Antibiotic-induced lung reaeration following 8-day treatment was measured separately in lung regions characterized by multiple and disseminated non confluent foci of pneumonia and in lung areas of confluent pneumonia. It was defined as the increase in gas volume in each lung region of interest following 8-day treatment.

**Figure 3** Computed tomography assessment of gas and tissue volumes in lung regions characterized by multiple and disseminated foci of pneumonia (left panels) and in lung areas of confluent pneumonia (right panels) at day 0 and 9, in patients treated by intravenous (open circles) and nebulized (closed circles) ceftazidime and amikacin. Figures 4A and 4B, show gas and tissue volumes changes in patients of aerosol and intravenous groups successfully treated by antimicrobial therapy. Figures 4C and 4D, show changes of gas and tissue volumes obtained in patients of aerosol and intravenous groups in whom antimicrobial therapy was unsuccessful.

**Figure 4** Computed tomography assessment of lung reaeration resulting from 8-day nebulization of ceftazidime and amikacin in a 53-year old patient with ventilator-associated pneumonia caused by susceptible *Pseudomonas aeruginosa*. VAP developed in the postoperative period of the surgical repair of a major thoraco-abdominal aneurysm. On the left side, 6 computerized tomography sections representative of both lungs and acquired before starting antibiotics are shown with
the corresponding color encoding system (normally aerated lung regions in dark grey, interstitial pneumonia and/or edema in light grey, foci of pneumonia, either disseminated or confluent, in red). On the right side, the same 6 computerized tomography sections acquired after 8-day nebulization of ceftazidime and amikacin are shown. Nebulization of antibiotics induced a 1000-ml lung reaeration, predominating in regions of confluent pneumonia.

**Figure 5** Computed tomography assessment of lung reaeration resulting from 8-day nebulization of ceftazidime and amikacin in a 32-year old patient with ventilator-associated pneumonia caused by *Pseudomonas aeruginosa* intermediate to ceftazidime (minimal inhibitory concentration of 12 mg.L\(^{-1}\)) and susceptible to amikacin complicating severe multiple trauma. On the left side, 6 computerized tomography sections, representative of both lungs and acquired before starting antibiotics are shown with the corresponding color encoding system (normally aerated lung regions in dark grey, interstitial pneumonia and/or edema in light grey, foci of pneumonia, either disseminated or confluent, in red). On the right side, the same 6 computerized tomography sections acquired after 8-day nebulization of ceftazidime and amikacin are shown. Nebulization of antibiotics induced a 930-ml lung reaeration, both in regions of non confluent foci of pneumonia and in regions of confluent pneumonia.
Table 1 Checklist form filled by nurse before and after each aerosol. Ventilator settings fixed for the nebulization period are: volume-controlled mode using constant inspiratory flow, respiratory frequency 12 breaths.min\(^{-1}\), inspiratory/expiratory ratio 50%, tidal volume 8 ml.kg\(^{-1}\) and end-inspiratory pause 20%.

<table>
<thead>
<tr>
<th>Medical orders</th>
<th>Physician __________ Date __________</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dosages</strong></td>
<td><strong>Ventilation before aerosol</strong></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>Mode ____</td>
</tr>
<tr>
<td>______ mg</td>
<td>RR _____/min</td>
</tr>
<tr>
<td>every 3 h</td>
<td>I/E ratio ____</td>
</tr>
<tr>
<td>Diluted in _____ ml</td>
<td>Plateau _____%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>TV_____ml</td>
</tr>
<tr>
<td>______ mg.day(^{-1})</td>
<td>FiO(_2) = ____ %</td>
</tr>
<tr>
<td>Diluted in _____ ml</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Checklist form</th>
<th>Nurse __________ Date __________</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>h</em>_ min</td>
<td><em>h</em>_ min</td>
</tr>
<tr>
<td>☐Cefta/AMK</td>
<td>☐Cefta/AMK</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Before aerosol</th>
<th>Before aerosol</th>
<th>Before aerosol</th>
</tr>
</thead>
</table>
| Removal of moisture exchanger | ☐          |             | ☐
| Removal of connecting tube     | ☐          | ☐           | ☐
| Nebulizer inserted 10 cm before Y piece | ☐          | ☐           | ☐
| Connection of expiratory filter positionned between expiratory circuit and ventilator | ☐          | ☐           | ☐
| Ventilator settings (see medical order) | ☐          | ☐           | ☐
| Patient desynchronized with the ventilator : start propofol | ☐          | ☐           | ☐

<table>
<thead>
<tr>
<th>After aerosol</th>
<th>After aerosol</th>
<th>After aerosol</th>
</tr>
</thead>
</table>
| Connection of moisture exchanger | ☐          | ☐           | ☐
| Reinsertion of connecting tube | ☐          | ☐           | ☐
| Removal of nebulizer | ☐          | ☐           | ☐
| Removal of expiratory filter | ☐          | ☐           | ☐
| Initial ventilator settings (see medical order) | ☐          | ☐           | ☐
| Stop propofol | ☐          | ☐           | ☐

Abbreviations: Cefta = ceftazidime; AMK = Amikacin, RR= Respiratory rate, TV= Tidal volume, I/E ratio = Inspiratory/Expiratory ratio
Table 2 Baseline clinical characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>Aerosol n=20</th>
<th>IV n=20</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>58 ± 15</td>
<td>60 ± 17</td>
<td>0.71</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>15(75)</td>
<td>18(90)</td>
<td>0.41</td>
</tr>
<tr>
<td>SAPS II, mean ± SD</td>
<td>33 ± 13</td>
<td>30 ± 10</td>
<td>0.47</td>
</tr>
<tr>
<td>SOFA, median (IQR)</td>
<td>3.5(2.5-7.5)</td>
<td>3.0(2.5-5.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>CPIS, median(IQR)</td>
<td>8(7-8)</td>
<td>9(8-9)</td>
<td>0.01</td>
</tr>
<tr>
<td>COPD, n (%)</td>
<td>3(15)</td>
<td>4(20)</td>
<td>1.00</td>
</tr>
<tr>
<td>Circulatory shock, n (%)</td>
<td>5(25)</td>
<td>2(10)</td>
<td>0.41</td>
</tr>
<tr>
<td>Admission category, n (%)</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Trauma</td>
<td>7 (35)</td>
<td>8 (40)</td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td>12 (60)</td>
<td>10 (50)</td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>1(5)</td>
<td>2(10)</td>
<td></td>
</tr>
<tr>
<td>Body temperature, mean ± SD</td>
<td>38.2 ± 0.6</td>
<td>38.5 ± 0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Leukocyte count, cell/mm³ mean ± SD</td>
<td>12470 ± 5582</td>
<td>13205 ± 5116</td>
<td>0.67</td>
</tr>
<tr>
<td>PaO₂/FiO₂, mean ± SD</td>
<td>266 ± 79</td>
<td>250 ± 66</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Abbreviations: SAPS II = Simplified Acute Physiology Score II; SOFA = Sequential Organ Failure Assessment; CPIS = Modified Clinical Pulmonary Infection Score; COPD = Chronic Obstructive Pulmonary Disease; SD = standard deviation; IQR = 25-75% interquartile range
Table 3  Antibiotic treatment efficiency.

<table>
<thead>
<tr>
<th></th>
<th>Aerosol n=20</th>
<th>Intravenous n=20</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cure of <em>P. aeruginosa</em> VAP at day 9 (n, %)</strong></td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>Day 9 : Positive BAL $\geq 10^4$ or miniBAL $\geq 10^3$ (n)</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Persisting <em>P. aeruginosa</em> VAP at day 9 (n, %)</td>
<td>3 (15%)</td>
<td>6 (30%)</td>
<td>0.26</td>
</tr>
<tr>
<td>VAP caused by superinfection at day 9 (n, %)</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>Recurrence of <em>P. aeruginosa</em> VAP (n)</td>
<td>3</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Recurrence of VAP caused by superinfection (n)</td>
<td>2</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of MV, median(IQR)</td>
<td>29(22-38)</td>
<td>18 (13-31)</td>
<td>0.13</td>
</tr>
<tr>
<td>Duration of MV after inclusion, median(IQR)</td>
<td>14 (7-22)</td>
<td>8 (6-12)</td>
<td>0.18</td>
</tr>
<tr>
<td>Length of stay in ICU, median(IQR)</td>
<td>38 (29-55)</td>
<td>29(18-44)</td>
<td>0.08</td>
</tr>
<tr>
<td>Length of stay in ICU after inclusion, median(IQR)</td>
<td>24 (18-48)</td>
<td>16 (11-23)</td>
<td>0.08</td>
</tr>
<tr>
<td>Mortality at day 28, n (%)</td>
<td>2(10%)</td>
<td>1(5%)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Cure of VAP = association of reduction of clinical and biological signs of infection, decrease in CPIS below 6, significant lung CT reaeration and lower respiratory tract specimens either sterile or with non-significant concentrations of *P. aeruginosa*. Persisting VAP = lack of improvement of clinical and biological signs, CPIS greater than 6, absence of CT lung reaeration with significant concentrations of *P. aeruginosa* persisting in lower respiratory tract specimens. Recurrence of *P. aeruginosa* VAP = initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of *P. aeruginosa* VAP. Superinfection = initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of VAP caused by pathogens other than *P. aeruginosa*.

IQR = Interquartile range, VAP = ventilator-associated pneumonia.
Table 4 Microbiological response to treatment and antibiotic susceptibility of *Pseudomonas aeruginosa* in each group of patients

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nebulized group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL (n)</td>
<td>20</td>
<td>17</td>
<td>16</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>BAL <em>P. aeruginosa</em> +</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5*</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> susceptibility (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAZ</td>
<td>AMK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>S</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>S</td>
<td>I †</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I ‡ S</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I § I †</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intravenous group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL (n)</td>
<td>20</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>BAL <em>P. aeruginosa</em> +</td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> susceptibility (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAZ</td>
<td>AMK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>S</td>
<td>17</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>S</td>
<td>I</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>S</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>S</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>I</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BAL = Bronchoalveolar lavage; *P. aeruginosa* = *Pseudomonas aeruginosa*; CAZ = ceftazidime; AMK = amikacin; S = Susceptible. *P. aeruginosa*’s susceptibility is defined as follows (36): for ceftazidime, S = Minimum inhibitory concentration (MIC) ≤ 4 mg.L\(^{-1}\); I = MIC > 4 and ≤ 32 mg.L\(^{-1}\); R = MIC > 32 mg.L\(^{-1}\); for amikacin, S = MIC ≤ 8 mg.L\(^{-1}\); I = MIC > 8 and ≤ 16 mg.L\(^{-1}\); R = >16 mg.L\(^{-1}\).

*In 2 patients, *P. aeruginosa* were identified at concentrations < 10\(^3\) cfu.mL\(^{-1}\) and there was no evidence of pneumonia recurrence (CPIS < 6 and improvement of lung aeration).

†MIC of amikacin = 16 mg.L\(^{-1}\); ‡MIC of ceftazidime = 12 and 32 mg.L\(^{-1}\); §MIC of ceftazidime = 6 mg.L\(^{-1}\).
Table 5 Amikacin and ceftazidime plasma concentrations measured on days 3 and 4

<table>
<thead>
<tr>
<th></th>
<th>Aerosol</th>
<th>IV</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ceftazidime</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dose (mg.kg(^{-1}))</td>
<td>76*</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>(C_{\text{peak}}) (mg.L(^{-1}))</td>
<td>12.1 ± 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C_{\text{trough}}) (mg L(^{-1}))</td>
<td>8.1 (6.0 -12.4)</td>
<td>32.2 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Amikacin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dose (mg.kg(^{-1}))</td>
<td>15.7*</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>(C_{\text{peak}}) (mg.L(^{-1}))</td>
<td>8.9 (5-11)</td>
<td>45.1 (33-58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(C_{\text{trough}}) (mg.L(^{-1}))</td>
<td>2.4 (1.7-5.9)</td>
<td>3.3 (1.9-5.8)</td>
<td>0.742</td>
</tr>
</tbody>
</table>

\(C_{\text{peak}}\) = Peak plasma concentrations measured 30 min after completion of nebulization or immediately at the end of the intravenous bolus administration; \(C_{\text{trough}}\) = Trough plasma concentrations measured immediately before the next nebulization, immediately before intravenous bolus administration or at any time in patients receiving continuous intravenous ceftazidime administration; IV = Intravenous; * = Dose reaching the respiratory system according to ceftazidime and amikacin extrapulmonary deposition. Data are expressed as mean±SD or median (25-75% interquartile range) according to data distribution.
Figure 1

46 patients: randomisation

Aerosol (n=24)  Intravenous (n=22)

4 excluded  2 excluded

Aerosol (n=20)  Intravenous (n=20)

day 1  

- cephalaxin 8 aerosols per day, 15 mg.kg⁻¹ each
- amikacin Single daily aerosol of 25 mg.kg⁻¹

intravenous

- cephalaxin (90 mg.kg⁻¹ per day or ciprofloxacin (strain) 400 mg x 2 per day
- amikacin 15 mg.kg⁻¹ per day or ciprofloxacin (strain) 400 mg x 2 per day

Day 1

Day 2  

Day 3

Day 4

Day 5

Day 7

Day 8

Day 9

Day 14

Day 28

Duration of treatment

Lung CT scan 1

BAL 1  

amikacin plasma concentrations

ceftazidime plasma concentrations

BAL 2

BAL 3

Lung CT scan 2

BAL 4

End of the study
Figure 2

1. Day 0 before antibiotic
2. Day 0
3. Day 0
4. Day 9 after antibiotic
5. Day 9
6. Day 9
Figure 3

A. Gas volumes in patients successfully treated by antimicrobial therapy

B. Tissue volumes in patients successfully treated by antimicrobial therapy

C. Gas volumes in patients with failure of antimicrobial therapy

D. Tissue volumes in patients with failure of antimicrobial therapy

NS

p = 0.004

p < 0.001

p < 0.001

p = 0.008

p < 0.024

p < 0.09

p < 0.001
Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*

Qin Lu, Jianxin Yang, Zhihai Liu, Claudia Gutierrez, Guy Aymard, Jean-Jacques Rouby and the Nebulized Antibiotics Study Group
MATERIAL AND METHODS

Study design and patients

The aim of the present study was to show that nebulization alone could cure VAP caused by *P. aeruginosa* and be at least as efficient as intravenous administration. Because of the absence of prior results of nebulization in patients with VAP caused by *P. aeruginosa*, we did not formally calculate a sample size for this pilot study.

The institutional review board of La Pitié-Salpêtrière approved the study protocol. Written informed consent was obtained from each patient or his next of kin and the study was conducted in two multidisciplinary intensive care units (ICU) of La Pitié-Salpêtrière hospital (Department of Anesthesiology and Critical Care Medicine, University Pierre et Marie Curie of Paris 6). Randomization was performed using a computer-generated randomization system. Ventilator-associated pneumonia was defined as the presence of new and persistent infiltrates on chest radiography associated with one of the following clinical features: (a) temperature ≥ 38.5°C or < 36.5°C, (b) leukocyte count > 10^4 µL^-1, (c) purulent bronchial secretions. *P. aeruginosa* was confirmed in lower respiratory tract specimens sampled either by fiberoptic bronchoscopy with non protected bronchoalveolar lavage (BAL) or protected minibronchoalveolar lavage (mini BAL) (E1). A positive sample was defined as ≥ 10^4 cfu.mL^-1 for non protected BAL and ≥ 10^3 cfu.mL^-1 for protected mini BAL (E2). Extrapulmonary infection (bacteremia, urinary infection, peritonitis, extrapulmonary abscesses, endocarditis etc…) was considered as exclusion criteria because it required the use of intravenous antibiotics. Among exclusion criteria, severe immunosuppression was defined as leukocyte count < 1000 cells.µL^-1 or
neutrophils < 500 cells.µL⁻¹ and resistance to ceftazidime and/or amikacin was defined as minimal inhibitory concentrations (MIC) > 32 mg.L⁻¹ for ceftazidime and MIC > 16 mg.L⁻¹ for amikacin according to the National Committee for Clinical Laboratory Standards (E3).

As previously recommended (E4-7), the rationale for determining nebulized and intravenous doses was to provide comparable amounts of ceftazidime and amikacin at the entry of the tracheobronchial tree (distal tip of the endotracheal tube) and at the entry of the pulmonary circulation (main pulmonary artery). Thereby, aerosol dose was determined as intravenous dose + extrapulmonary deposition. Extrapulmonary deposition, measured in an in vitro preliminary study, was found at 30% for ceftazidime and 40% for amikacin, values that served for determination of nebulized doses.

In the aerosol group, patients received 8 aerosols per day of nebulized ceftazidime for 8 days and a single daily aerosol of nebulized amikacin for 3 days. We hypothesized that the high lung tissue concentrations generated by combined ceftazidime and amikacin during the first 3 days of treatment was a determinant factor for successful clinical cure. As a consequence, ceftazidime and amikacin aerosols were maintained for at least 3 days and weaning test was allowed only after this period of full treatment. If _P aeruginosa_ was intermediate to ceftazidime (MIC 4–32 mg.L⁻¹) and/or amikacin (MIC 8–16 mg.L⁻¹) but susceptible to ciprofloxacin, the patients in the aerosol group received nebulized ceftazidime and amikacin whereas in patients of the intravenous group, ceftazidime or amikacin was replaced by ciprofloxacin (400 mg.12h⁻¹). The duration of the antimicrobial treatment was 8 days in both groups of patients.
Bedside chest radiographies and leukocyte count were performed daily. Lower respiratory tract specimens (BAL and mini BAL) were collected in mechanically ventilated patients on day 3, day 5, day 7, day 9 following the start date of the treatment. In the aerosol group, lower respiratory tract specimens were sampled 2.5 hours following a ceftazidime aerosol. Blood cultures were sampled at baseline, day 1 and day 2 and then according to the clinical signs (fever $\geq 38.5 \, ^{\circ}C$ or $\leq 36.5 \, ^{\circ}C$ or shivering). A thoracic computed tomography scan was acquired the day before starting antibiotics (day 0), and at the end of the treatment (day 9). Modified clinical pulmonary infection score (CPIS) (E8) were determined at day 0, day 3, day 5, day 7 and day 9.

**Response to antibiotic treatment**

On day 9, response to antibiotic treatment was classified by non blinded investigators in 4 categories: 1) “Cure” of VAP, defined as the association of reduction of clinical and biological signs of infection, decrease in CPIS below 6, significant lung CT reaeration, and lower respiratory tract specimens either sterile or with non-significant concentrations of *P aeruginosa*. 2) Persisting VAP, defined as lack of improvement of clinical and biological signs, CPIS greater than 6, absence of CT lung reaeration with significant concentrations of *P aeruginosa* persisting in lower respiratory tract specimens. 3) Recurrence of *P aeruginosa* VAP was defined as initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of *P aeruginosa* VAP. 4) Superinfection was defined as initial cure after 8 days of antimicrobial therapy followed by post-treatment relapse of VAP caused by pathogens other than *P aeruginosa*. 
Aerosol generation

The vibrating plate nebulizer (Aeroneb Pro®, Aerogen Nektar Corporation, Galway, Ireland) was positioned on the inspiratory limb 10 cm proximal to the Y-piece (9). After inserting into the chamber of the nebulizer 15 mg/kg of ceftazidime powder diluted in 10 ml of sterile water or 25 mg/kg of amikacin powder diluted in 15 ml, each nebulization was delivered over 30 to 60 min according to the dosage. Specific ventilator settings were used during the nebulization period to reduce as much as possible flow turbulences and, thereby, extrapulmonary deposition: removal of heat and moisture exchanger or conventional humidifier, volume controlled mode using a constant inspiratory flow, respiratory rate of 12 breaths.min\(^{-1}\), inspiratory/expiratory ratio of 50%, tidal volume of 8 ml.kg\(^{-1}\), end-inspiratory pause representing 20% of the duty cycle. During the nebulization period, expired aerosolized particles with pore size ≥ 0.2 µm were collected in a filter positioned on the distal part of the expiratory limb (Hygrobac; Mallinckrodt Medical, Mirandola, Italy). After each aerosol, the filter was removed and heat and moisture exchanger or conventional humidifier repositioned. During the nebulization period, strict coordination between the patient and the ventilator was required in order to avoid inspiratory turbulences and optimize distal lung deposition of aerosolized particles. In presence of discoordination, 2 mg.kg\(^{-1}\) of propofol was infused.

In 17 patients, extrapulmonary deposition was measured as previously described (E9): after completion of a single nebulization of ceftazidime and amikacin, nebulization chamber, inspiratory circuit and expiratory filter were rinsed respectively in a fixed 1-liter volume of distilled water to assess ceftazidime and amikacin
concentrations present in respiratory circuits. Percentage of total extrapulmonary deposition was calculated as the amount of ceftazidime or amikacin recovered in the different respiratory circuits divided by the dose of ceftazidime and amikacin inserted in the nebulizer chamber. Percentage of ceftazidime and amikacin entering the respiratory system was defined as 100 minus percentage of extrapulmonary deposition.

**Computed Tomography Measurements**

Each patient was transported to the Department of Radiology by two physicians. Spiral CT sections were acquired using a fast spiral Brilliance™ CT v3.0.0.19206 (Phillips, Eindhoven, The Netherlands) at day 0 (immediately before starting antimicrobial therapy) and day 9 (after 8-day administration of intravenous or nebulized ceftazidime and amikacin). To provide similar conditions of measurements at day 0 and day 9 a time at which nearly half of the patients were spontaneously breathing, contiguous axial 5-mm thick CT sections were acquired from the apex to the diaphragm (E10) in the supine position at zero end-expiratory pressure. Mechanically ventilated patients were anesthetized and paralyzed for transportation to the Department of Radiology. CT sections were acquired during a 7-second disconnection from the ventilator. Spontaneously breathing patients were asked to hold their breath at end-expiration for 7 seconds for CT acquisition.

**Measurement of pulmonary volumes and lung aeration**

CT sections were reconstructed from the volumetric data using standard filter in order to avoid artifactual changes in poorly-, non aerated and hyperinflated
compartments (E11). CT data were analyzed using a specifically designed software (Lungview, Institut National des Télécommunications, France) including a color coding system (E12). Volumes of gas and tissue, total lung volume were computed from the following equations (E13), in which CT number is the CT attenuation of the compartment analyzed:

\[
\text{volume of the voxel} = (\text{size of the pixel})^2 \times \text{section thickness (1)}
\]

\[
\text{total lung volume} = \text{number of voxels} \times \text{volume of the voxel (2)}
\]

\[
\text{volume of gas} = (-\text{CT number}/1,000) \times \text{total volume}, \text{if the compartment considered has a CT number below 0 (volume of gas} = 0 \text{if the compartment considered has a CT number above 0)} (3),
\]

\[
\text{volume of lung tissue} = (1 + \text{CT number}/1,000) \times \text{total volume}, \text{if the compartment considered has a CT number below zero (4), volume of lung tissue} = \text{number of voxels} \times \text{volume of the voxel}, \text{if the compartment considered has a CT number above zero (5)}.
\]

**Measurement of lung reaeration resulting from nebulized and intravenous antibiotics**

Gas and tissue volumes were measured at day 0 and day 9. Antibiotic-induced lung reaeration was measured on each CT section as the increase in gas volume in lung regions characterized by multiple and disseminated foci of pneumonia and in lung areas of confluent bronchopneumonia following 8-day treatment. As recently
described (E14), reaeration following successful treatment of VAP is observed not only in lung regions with non confluent foci of pneumonia but in lung areas of confluent bronchopneumonia. To assess the distribution of lung reaeration, a method based on regional analysis and derived from the method proposed by Malbouisson et al. (E15) for measuring positive end-expiratory pressure-induced lung recruitment, was used. First, 2 CT sections acquired at day 0 and 9, and corresponding to the same anatomical level, were simultaneously displayed on the screen of the computer. The color encoding system integrated in the Lungview® software served to separate two regions of interest on each CT section: regions with non confluent foci of pneumonia and regions of confluent bronchopneumonia. As previously described (E14), regions with non confluent foci of pneumonia were made of disseminated rounded CT attenuations separated by normally aerated lung areas and regions of confluent bronchopneumonia were made of CT consolidations predominating in lower lobes. In a second step, by referring to anatomical landmarks, the limit between the two regions of interest delineated on the CT section at day 0 was manually redrawn on the CT section obtained at day 9. Regional antibiotic-induced lung reaeration was computed as the amount of gas which penetrated within each region of interest between day 0 and day 9. This time-consuming analysis was repeated on each pair of CT sections and total lung reaeration was computed as the sum of each regional lung reaeration. In some patients who had a massive lung reaeration following antimicrobial therapy, two CT sections obtained at day 9 corresponded to a single CT section obtained at day 0, as attested by the anatomical landmarks (divisions of bronchial and pulmonary vessels). In such a situation, the region of interest manually delineated on the CT section obtained at day 0 was manually redrawn twice on each of the 2 CT sections obtained at day 9. Decrease in
lung inflammation resulting from antimicrobial agents was measured as the total decrease in lung tissue volume between day 0 and day 9.

**Measurements of ceftazidime and amikacin plasma concentrations**

Ceftazidime plasma concentrations were measured at day 4. In patients treated by intravenous ceftazidime, trough plasma concentrations were measured on blood samples obtained at day 4. In patients treated by nebulized ceftazidime, peak and trough plasma concentrations were measured respectively 30 min after completion of nebulization and immediately before the next nebulization. Ceftazidime plasma concentrations were measured using high-performance liquid chromatography method (E16).

Amikacin plasma concentrations were measured at day 3. Peak and trough plasma concentrations were measured respectively on blood samples drawn at the end of the single daily administration and 24h later. Amikacin plasma concentrations were measured using a fluorescence polarization immunoassays (FPIA) method (TDx; Abbot Laboratoires, Abott Park, IL). All blood samples were immediately centrifuged for 15 min (4000 g) at 4 °C and plasma samples were stored at -40 °C for later analysis.

**Safety assessment**

Safety evaluation included medical history, physical examinations, measurement of vital signs and standard laboratory tests. Adverse events such as bronchospasm and hypoxemia were specifically notified. Blood gases were sampled in each patient before and after the first aerosol. Adverse events were categorized
according to the Medical Dictionary for Regulatory Activities System and reported for both groups.

**Statistical analysis**

The sample size was planned to have approximately 20 patients in each group, to provide initial safety assessment and efficacy estimates in this pilot phase II trial.

Data are expressed as mean ± SD or as median and 25%-75% interquartile range (IQR) according to data distribution. Quantitative data of baseline characteristics and clinical outcome were compared between aerosol and intravenous groups using bilateral unpaired Student’s test or Mann-Whitney rank-sum test according to data distribution. Nominal data were compared by Pearson’s chi-square test or a Fisher’s exact test. Evolution of clinical signs and CT changes of gas and tissue volumes were compared between the two groups by two-way ANOVA for repeated measure. Statistical analysis was performed using SPSS 13.0 and SigmaStat 2.03 (SPSS, San Rafael, CA). The statistical significance level was fixed at 0.05.

**RESULTS**

**Antibiotic treatment efficacy**

Evolution of temperature and Clinical Pulmonary Infection Score of both groups following antimicrobial therapy is shown in figure E1.
REFERENCES


E3. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: eleventh informational supplement, Wayne, PA. *NCCLS* 2001; M100-S111.


E8. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and


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Figure E1. Evolution of body temperature and Clinical Pulmonary Infection Score (CPIS) between day 0 and day 9 in patients of both groups in whom antimicrobial therapy was successful or failed (Closed circles = Patients successfully treated by antimicrobial therapy; Open circles = Patients with failure of antimicrobial therapy)
Figure E1

**Aerosol**
- Temperature vs. days after inclusion:
  - Days 0, 3, 5, 7, 9
  - Temperature range: 37.2°C to 38.8°C

**Intravenous**
- Temperature vs. days after inclusion:
  - Days 0, 3, 5, 7, 9
  - Temperature range: 37.4°C to 38.6°C

**Aerosol**
- CPIS vs. days after inclusion:
  - Days 0, 3, 5, 7, 9
  - CPIS range: 3 to 11

**Intravenous**
- CPIS vs. days after inclusion:
  - Days 0, 3, 5, 7, 9
  - CPIS range: 4 to 10