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Influence of positive end-expiratory pressure (PEEP) on histopathological and bacteriological aspects of pneumonia during low tidal volume mechanical ventilation

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Abstract Objective: Ventilatory strategies combining low tidal volume (V_T) with positive end-expiratory pressure (PEEP) are considered to be lung protective. The influence of the PEEP level was investigated on bacteriology and histology in a model of ventilator-associated pneumonia. **Subjects:** Nineteen New Zealand rabbits. **Interventions:** The animals were mechanically ventilated with a positive inspiratory pressure of 15 cmH₂O and received either a zero end-expiratory pressure (ZEEP, $n=6$), a 5 cmH₂O PEEP ($n=5$) or a 10 cmH₂O PEEP ($n=4$). An inoculum of *Enterobacter aerogenes* was then instilled intrabronchially. The non-ventilated pneumonia group ($n=4$) was composed of spontaneously breathing animals which received the same inoculum. Pneumonia was assessed 24 h later. **Main results:** The lung bacterial burden was higher in mechanically ventilated animals compared with spontaneously breathing animals. All animals from

the latter group had negative spleen cultures. The spleen bacterial concentration was found to be lower in the 5 cmH₂O PEEP group when compared to the ZEEP and 10 cmH₂O PEEP groups (3.1 ± 1.5 vs 4.9 ± 1.1 and 5.0 ± 1.3 log₁₀ cfu/g, respectively; $p < 0.05$). Lung weight and histological score values were lower in the spontaneously breathing animals as well as in the 5 cmH₂O PEEP group compared with the ZEEP and 10 cmH₂O groups. **Conclusions:** Mechanical ventilation substantially increased the lung bacterial burden and worsened the histological aspects of pneumonia in this rabbit model. Variations in terms of lung injury and systemic spreading of infection were noted with respect to the ventilatory strategy.

Keywords Ventilator-associated pneumonia · Animal model · Ventilation-induced lung injury · Positive end-expiratory pressure

Introduction

Recently, an increasing number of experimental studies have emphasized the potential for mechanical ventilation (MV) to cause lung damage, termed ventilator-induced lung injury (VILI) [1]. Pre-existing tissue injury is thought to work together with MV to cause lung damage [2]. Both lung over-distension and the cyclic closing and opening of lung terminal units appear to play a role in the development of VILI. Ample experimental data have now

shown that ventilation strategies combining low tidal volume (V_T) with positive end-expiratory pressure (PEEP) to keep the lung open without over-distending it were 'lung-protective'. These experimental findings have recently been translated into clinical practice where the use of such strategies has been shown to decrease the systemic consequences of injurious ventilation as well as mortality in ventilated critically ill patients. [3, 4]. However, selecting the right level of PEEP in such patients remains controversial [5, 6].

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection observed among patients undergoing MV. Increased mortality and length of stay are generally associated with the occurrence of VAP [7, 8]. Therefore, among experimental studies which aim at evaluating the influence of the ventilation strategy on lung injury, those in which the lung insult is a bacterial challenge are of particular interest. Several authors have reported that an injurious ventilatory strategy combining a large tidal volume with a modest level of PEEP worsened lung injury, increased the lung bacterial burden and enhanced the systemic spread of infection [9–13]. However, the beneficial effect of adding PEEP during experimental pneumonia was sought only in animals submitted to high V_T . Conflicting results regarding the effect of PEEP have been reported in non-infectious lung injury animal models [14–17].

We have previously described a model of VAP in rabbits [18]. Our findings suggested that MV was likely to cause VILI in spite of using a protective ventilation strategy (e.g., low V_T plus low PEEP), provided the animals were concurrently submitted to a bacterial challenge. The present study, therefore, was designed to assess to what extent the level of PEEP could influence both the histological and bacteriological parameters of our rabbit VAP model.

Materials and methods

Animals

Male New Zealand white rabbits (body weight 2.6–3.3 kg) were obtained from Elevage scientifique des Dombes (Romans, France). These animals were not immunosuppressed and had a sanitary status of virus antibody free and specific pathogen free. They were placed in individual cages and were nourished ad libitum with drinking water and food, according to National Institutes of Health guidelines. The study was approved by our institutional review board for the care of animal subjects.

A central venous catheter was surgically inserted 24 h before MV began as previously described [19]. MV was not required at this time.

Mechanical ventilation

Under general anesthesia provided by iterative intravenous injections of propofol (Rapinovet, Schering-Plough), the rabbit was orally intubated before being connected to a pressure-controlled ventilator (RPR). MV was performed in the supine position with a continuous infusion of ketamine (Imalgene, Rhône-Poulenc, 1 mg/kg per h) and pancuronium bromide (Pavulon, Organon-Teknika, 0.3 mg/kg per h). During the MV period, the animals were hydrated by infusing i.v. 150 cc/kg per day of isotonic serum.

Positive inspiratory pressure (PIP) was adjusted to approximately 15 cmH₂O, in order to deliver a V_T of 8 cc/kg at zero end-expiratory pressure (ZEEP). The V_T was measured at the onset of the MV by placing a pneumotachometer between the ventilator circuit and the endotracheal tube. The animals were then randomly assigned to receive either a ZEEP (ZEEP group), a 5 cmH₂O PEEP (low PEEP group [lPEEP]) or a 10 cmH₂O (high PEEP group

[hPEEP]), whereas PIP was kept constant throughout the experiment. The other ventilator settings were a fraction of inspired oxygen (FIO₂) of 0.5 and a respiratory rate of 30 breaths/min with 30% inspiratory time.

Arterial blood gases and hemodynamic parameters were monitored in several test rabbits. These parameters appeared to be stable throughout the experiment, regardless of the levels of PEEP applied. Therefore, invasive monitoring was not systematically performed. Air-breathing infected animals were used as controls (non-ventilated pneumonia group [NVP]).

Bacterial challenge

The modalities of bacterial challenge have been described elsewhere [18]. Briefly, a clinical strain of *Enterobacter aerogenes* was used to prepare a calibrated inoculum. A $10.5 \pm 0.4 \log_{10}$ cfu/ml mean titer was used in this study.

After 1 h of MV, a silicon catheter was introduced through the endotracheal tube and pushed until it reached the bronchi. Then, 0.5 ml of a freshly prepared inoculum was gently flushed through this catheter. Thereafter, a 5 cc air volume was rapidly flushed into the airways as a recruitment maneuver just before the animal was connected again to the ventilator. FIO₂ was transiently set to 1.0 within the time of inoculation. MV was then continued for 24 h. Notably, tracheal aspirates were never suctioned during the experimental course. Therefore, no additional recruitment maneuver was needed.

In animals from the NVP group, an endobronchial silicon catheter was introduced orally under visual control as previously described [19]. Similarly, 0.5 ml of inoculum was flushed through this catheter, which was immediately removed. The animals were placed back in their cages with free access to food and water.

Pneumonia evaluation

All rabbits were anesthetized and then killed by an overdose of thiopental 24 h after the inoculation. Autopsies were carried out and the lungs and spleen were aseptically taken, and the lungs were exsanguinated. The presence of pneumonia was assessed based on both histological and microbiological findings, as follows.

Assessment of pneumonia

Lung injury evaluation was based on the microscopic examination. A 1 cm³ sample was taken from the worst macroscopic lesion from all lobes for microscopic examination. In the absence of macroscopic abnormality within the lobe, a lung fragment was sampled arbitrarily. A pathologist, unaware of both the ventilation strategy and the macroscopic and microbiological data, performed the histological evaluation. Each specimen was graded using a histopathological score grid used to grade pneumonia in humans [20]. The worst lesion of each lobe was graded in six categories: normal (0 point), vascular injury (1 point), bronchiolitis (2 points), focal pneumonia (3 points), confluent pneumonia (4 points) and abscessed pneumonia (5 points). The overall score, corresponding to the sum of lobar score values, ranged from 0 to 35 points. In addition, aspects of diffused alveolar damage (DAD), including hyaline membrane formation (HMF), as well as emphysema-like lesions were sought in each lobe. Thus, the number of lobes in which such abnormalities were found was noted in each rabbit.

Microbiological evaluation of pneumonia

After sampling for histological examination, each pulmonary lobe was isolated from the whole lung, homogenized in sterile water and used for serial tenfold dilution cultures. The bacterial concentration value for each lobe was adjusted to its weight. The mean bacterial pulmonary concentration was calculated according to each lobar concentration with lobar weight (e.g., mean concentration = Σ [lobar concentration x lobar weight] / Σ lobar weights). In addition, the spleen of each rabbit was removed, weighed, homogenized and cultured. An *E. aerogenes* positive spleen culture was considered as a marker of systemic bacterial spreading.

Statistical analysis

Data are presented as means \pm SD, unless otherwise specified. Comparisons of values between the groups were assessed both by one-way analysis of variance (using the ventilatory strategy as blocking factor) completed by a post hoc analysis using the Student-Newman-Keuls test, and Kruskal-Wallis test. A *p* value of less than 0.05 was considered to indicate statistical significance. All calculations were performed with Statview software (SAS Institute, Cary, NC, USA).

Results

Population

A total of 19 rabbits were studied and the different experimental groups were as follows: IPEEP (*n*=5); ZEEP (*n*=6); hPEEP (*n*=4); NVP (*n*=4). The mean body weight was 2.9 ± 0.2 kg, without any significant difference between groups (2.9 ± 0.2 , 2.9 ± 0.2 , 2.8 ± 0.1 and 2.8 ± 0.2 , respectively; *p*=0.75).

It should be noted that the pneumonia was never found to be lethal. Three animals in the hPEEP group died early from compressive pneumothoraces and were subsequently replaced.

Bacteriological findings

Quantitative lung cultures showed a high mean concentration of *E. aerogenes* in the four groups 24 h after inoculation (Fig. 1). The bacterial lung burden was found to be significantly lower in spontaneously breathing control animals than in ventilated rabbits, when all three groups were considered (5.1 ± 0.5 \log_{10} cfu/g vs 6.8 ± 0.7 \log_{10} cfu/g, respectively; *p*=0.0005). No significant difference in terms of bacterial lung burden was found between hPEEP, IPEEP and ZEEP groups (7.3 ± 0.8 \log_{10} cfu/g vs 6.5 ± 0.8 \log_{10} cfu/g and 6.8 ± 0.4 \log_{10} cfu/g, respectively; *p*=0.54).

Based on spleen cultures performed 24 h after inoculation, the rate of bacteremia was high in the three groups that had undergone MV, whereas none of the air-breathing animals were found to have positive spleen cultures (Fig. 2). Interestingly, adding a low PEEP level was as-

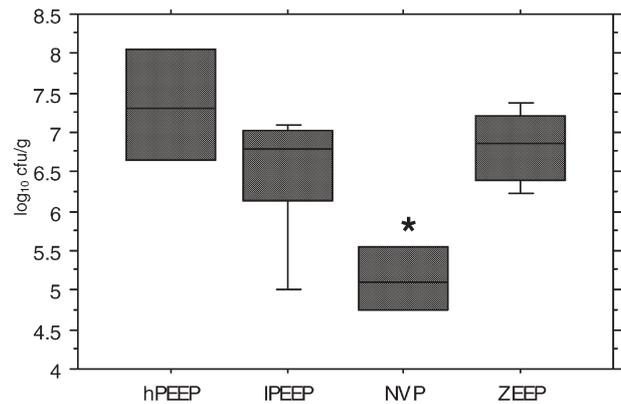


Fig. 1 Comparison of the mean lung bacterial concentrations (\log_{10} cfu/g of tissue) of rabbits 24 h after bacterial challenge with respect to the ventilation strategy (ZEEP, IPEEP or hPEEP). Infected air-breathing animals were used as controls (NVP). *statistical significance between NVP and other groups; hPEEP low V_T , PEEP =10 cmH_2O , IPEEP low V_T , PEEP =5 cmH_2O , ZEEP low V_T , PEEP =0 cmH_2O , NVP non-ventilated pneumonia

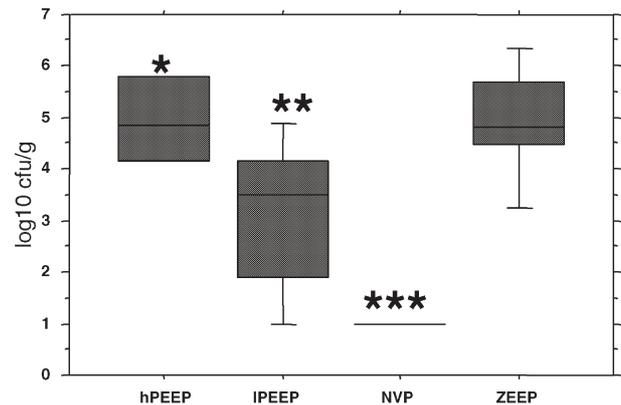


Fig. 2 Comparison of the mean spleen bacterial concentrations (\log_{10} cfu/g of tissue) of rabbits 24 h after bacterial challenge with respect to the ventilation strategy (ZEEP, IPEEP or hPEEP). Infected air-breathing animals were used as controls (NVP). *statistical significance between IPEEP and hPEEP groups, **statistical significance between IPEEP and ZEEP groups, ***statistical significance between NVP and all three other groups; hPEEP low V_T , PEEP =10 cmH_2O , IPEEP low V_T , PEEP =5 cmH_2O , ZEEP low V_T , PEEP =0 cmH_2O , NVP non-ventilated pneumonia

sociated with reduced pulmonary-to-systemic translocation, since spleen cultures exhibited significantly lower bacterial concentrations in this group compared to the ZEEP group (3.1 ± 1.5 \log_{10} cfu/g vs 4.9 ± 1.1 \log_{10} cfu/g, respectively; *p*=0.025). The further increase in the PEEP level from 5 to 10 cmH_2O was not associated with a reduction in the concentration of spleen bacteria, but rather with a significantly increased bacterial burden when compared with the IPEEP group (5.0 ± 1.3 \log_{10} cfu/g vs 3.1 ± 1.5 \log_{10} cfu/g, respectively; *p*=0.032).

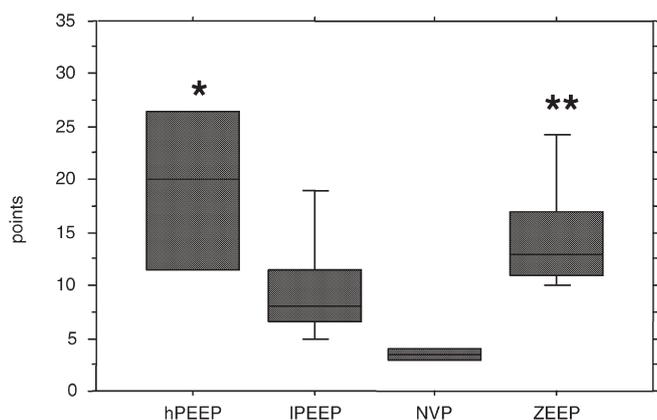


Fig. 3 Comparison of the microscopic score value (points) of rabbits 24 h after bacterial challenge with respect to the ventilation strategy (ZEEP, IPEEP or hPEEP). Infected air-breathing animals were used as controls (NVP). *statistical significance between hPEEP and both IPEEP and NVP groups, **statistical significance between ZEEP and NVP groups, *hPEEP* low V_T , PEEP =10 cmH₂O, *IPEEP* low V_T , PEEP =5 cmH₂O, *ZEEP* low V_T , PEEP =0 cmH₂O, *NVP* non-ventilated pneumonia

Histological findings

Gross examination

Although the lungs of the hPEEP group animals appeared to be more congested than those from the other groups, animals from all groups had similar gross evidence of consolidating pneumonia in at least two distinct lobes, irrespective of the ventilatory strategy.

Microscopic examination

Histology revealed signs of pneumonia in all animals. Polymorphonuclear leukocytes and fibrinous exudate filling up the alveoli were observed in all specimens. A higher level of lung injury was found in mechanically ventilated, compared to spontaneously breathing rabbits (Fig. 3). Differences were noted within mechanically ventilated animal groups with regard to the degree of lung injury. Lung injury tended to be less intense when a 5 cmH₂O PEEP was applied compared with ZEEP (9.6±5.5 vs 14.8±5.5 points, respectively; $p=0.15$). Interestingly, a further increase in the PEEP level (from 5 to

10 cmH₂O) significantly increased the histology lung injury score, from 9.6±5.5 to 19.0±8.9 points, respectively ($p=0.030$).

Differences were noted with regard to the presence of DAD, as well as emphysema-like lesions (Table 1, Fig. 4). None of these features were found in the lungs from spontaneously breathing animals. In contrast, DAD features coexisted with pneumonia in the lungs of the ZEEP group animals. The adjunction of a low PEEP seemed to reduce the occurrence of DAD. In the 10 cmH₂O PEEP group, emphysema-like lesions were constantly encountered in the lobes free of pneumonia.

Discussion

The main findings of the present study are the following: (1) MV dramatically worsened the histological features of pneumonia, caused additional diffuse alveolar damage, and promoted local bacterial growth and systemic translocation, compared to spontaneous negative-pressure ventilation; (2) during MV, the level of PEEP could alter the degree of lung injury as well as the systemic spreading of the infection.

Positive pressure ventilation in anesthetized animals has been shown to be potentially deleterious per se, even when low V_{TS} are used [21, 22]. It is hypothesized that MV-induced cellular stretching is able to trigger an inflammatory reaction within the airspace and that a second insult, such as a bacterial challenge, is necessary for the inflammation/lung injury to become 'clinically relevant' [2, 18, 23, 24]. However, the links between such a pro-inflammatory lung response and the MV-induced increased severity of pneumonia have not yet been established. Lung bacterial clearance may be impaired by MV through the induction of a local environment favorable to bacterial overgrowth.

Furthermore, MV may impair bacterial clearance by other mechanisms, such as an impairment of cough and mucociliary clearance due to anesthesia, the supine positioning and the presence of an endotracheal tube. The resulting increase in the lung burden could account for the MV-induced pulmonary-to-systemic bacterial translocation which has been reported in various animal models [9–13]. This may also be related to an increased injury of the alveolar-capillary barrier and/or the physical effect of positive pressure "pushing" bacteria out of the airways. In

Table 1 Mean number of lobes per rabbit in which microscopic examination revealed non-infectious lung injury according to the ventilation strategy (ZEEP, IPEEP or hPEEP). Infected spontaneously-breathing animals were used as controls (NVP)

	Study groups			
	hPEEP (n=4)	IPEEP (n=5)	ZEEP (n=6)	NVP (n=4)
Emphysema-like lesions	1.2 (1.9)	0.0 (0.0)	0.8 (0.3)	0.0 (0.0)
Diffused alveolar damage	1.5 (1.9)	0.2 (0.4)	0.3 (0.8)	0.0 (0.0)

hPEEP low tidal volume (V_T), PEEP =10 cmH₂O, *IPEEP* low V_T , PEEP =5 cmH₂O, *ZEEP* low V_T , PEEP =0 cmH₂O, *NVP* non-ventilated pneumonia

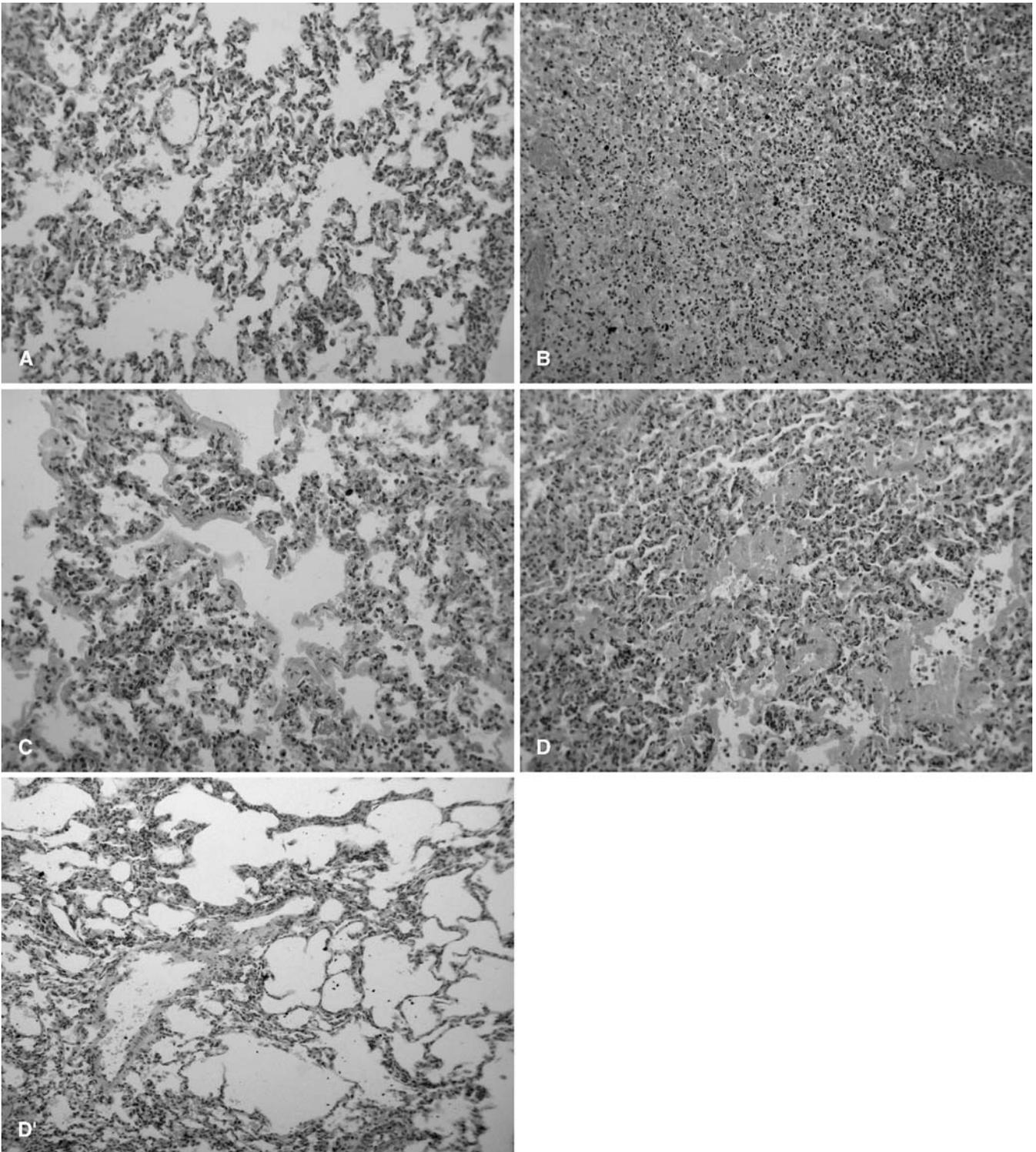


Fig. 4 Lung histopathological examination of *E. aerogenes* pneumonia. **A** NVP group: polymorphonuclear leukocytes infiltrate within the alveolar wall **B** ZEEP group: polymorphonuclear leukocytes infiltrate filling up alveoli with late-stage diffused alveolar damage **C** IPEEP group: hyaline membrane formation reflecting early-stage diffused alveolar damage **D** hPEEP group:

advanced diffused alveolar damage coexisting with pneumonia features **D'** hPEEP group: emphysema-like lesions in the lung region without pneumonia. Hematoxylin-eosin-safran stain was applied to the sections. Original magnification: X 100. *NVP* non-ventilated pneumonia, *hPEEP* low V_T , PEEP = 10 cmH₂O, *IPEEP* low V_T , PEEP = 5 cmH₂O, *ZEEP* low V_T , PEEP = 0 cmH₂O

the present study, we found that the rabbits submitted to MV exhibited significantly greater lung bacterial concentrations as well as more injury when compared to the spontaneously breathing ones. Therefore, we conclude that both mechanisms could account for the extra-pulmonary bacterial dissemination in the setting of our animal model of VAP. In addition, our data highlight the potential role of VILI in this context since microscopic examination of lung samples showed HMF only in the lungs submitted to MV.

Since it has been shown that the addition of PEEP to low V_T MV could be beneficial, the question of the effect of various levels of PEEP in the setting of pneumonia was also addressed in the present study [25, 26]. Added PEEP has been found to be protective in animal models of VAP, resulting in decreased lung injury and bacterial pulmonary-to-circulation translocation [9–13]. However, in these studies, the animals were subjected to lung overdistension since V_T and/or PIP was high. Therefore, the addition of PEEP may have been protective by lessening the lung damage caused by a deleterious ventilatory regimen, as previously demonstrated [27–29]. In addition, the ventilatory regimens tested were very different from those currently used in clinical practice [5].

Our results provide new information on the effect of varying PEEP levels in a ventilatory setting relevant to that used in patients with acute lung injury in intermediate-sized animals. Although the lung bacterial burden was not influenced by the PEEP settings, as previously found, a low level of PEEP was associated with reduced systemic translocation when compared to ZEEP [26]. Although our results do not allow us to draw clear conclusions regarding this point, our histological data provide some interesting clues. We observed that adding 5 cmH₂O PEEP was associated with a trend toward a reduction in lung injury in animals with pneumonia ventilated with a low tidal volume. Such findings could illustrate how PEEP reduces pulmonary-to-systemic bacteria translocation by preventing VILI in infected lung areas, as suggested elsewhere [26, 30]. Although speculative, other mechanisms could be considered. Thus, PEEP has been shown to reduce lung edema, to allow surfactant preservation and to redirect the blood flow into poorly aerated alveoli (e.g., infected lung areas) [1]. In addition, PEEP could have reduced the spleen perfusion. However, since we used a pressure-controlled ventilator, the three groups were not submitted to the same V_{TS} and this may account for the differences observed in terms of lung injury. Therefore, the V_T reduction subsequent to the addition of 5 cmH₂O of PEEP could account, at least in part, for its benefit.

When a 10 cmH₂O PEEP was applied (hPEEP group), significantly more bacteria were recovered from spleen cultures than in the IPEEP group. Concurrently, lung injury including pneumonia severity and emphysema-like lesions in non-pneumonic regions tended to be greater in

the hPEEP than in the IPEEP group, possibly reflecting the extent to which the well-aerated lung regions were inflated, whereas poorly aerated alveoli could not be recruited because of the pneumonic lung stiffness. Our findings would be in accordance with the results of studies that attempted to assess lung aeration distribution in humans with ARDS. Indeed, some authors have shown that when lung injury was primary, i.e. aspiration or bacterial pneumonia, the addition of high PEEP could be deleterious since its application resulted in overinflation of the normally aerated lungs rather than in recruitment of the remaining poorly aerated lung [6, 31, 32].

Finally, some experimental studies have suggested that high PEEP levels might attenuate lung inflammation, resulting in a reduction in bacterial clearance [29, 33, 34]. This could, at least in part, be explained by the sequestration of polymorphonuclear leukocytes within the alveolar capillaries by PEEP [35]. The assessment of inflammatory cells and biomarkers was not performed in the present study and we could therefore not corroborate these findings.

There are some limitations of our model. First, experimental results should be taken cautiously, especially when small animal species are studied. For example, small animals seem to be more prone to VILI and bacteria translocation from the lung than larger animals [1]. Second, the fact that the randomization to the different PEEP levels occurred before bacterial instillation could be questioned and may account for the different rates of pulmonary-to-systemic translocation, due to variable bacteria distribution. We tried to minimize this putative effect by performing a similar recruitment maneuver following the bacterial inoculation in animals from all PEEP groups. In addition, no significant difference was found between these groups in terms of lobar bacterial concentrations (data not shown). Third, since invasive monitoring was not used, one cannot rule out hemodynamic modifications that may have played a role in the systemic spread of the infection and in the ventilator-induced diffuse alveolar damage. Fourth, V_T may have changed during the 24-h period of ventilation, due to changes in the lung mechanical properties in relation to the development of pneumonia. Therefore, the direct effect of PEEP on the pneumonia features cannot be ascertained. Fifth, since neither PaCO₂ nor arterial blood pH was monitored, an effect of hypercapnic acidosis on lung injury cannot be excluded. However, greater PaCO₂ values should be expected in the hPEEP group when compared to the IPEEP group, assuming that V_T was lower in the former, that could in turn be lung protective [34].

In conclusion, our results suggest that the addition of PEEP is probably helpful in animals with bacterial pneumonia and submitted to a low V_T [36]. However, the end-expiratory volume, if excessive, could be an important determinant of the degree of lung injury, as previously described in non-infected animals, which could, in

turn, influence the systemic spreading of the infectious process [37]. These results should be taken as a word of caution by the clinician when setting the PEEP level in patients with pneumonia who require MV. It would be of

interest to investigate in further in vivo or in vitro studies whether over-distension per se may impair local lung and/or systemic immune defense against bacterial infection.

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