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Title: High tidal volume ventilation does not exacerbate acid-induced lung injury in infant rats

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Abstract (160 words)

The impact of high $V_T$-low PEEP in infant rats with preinjured lungs is unknown. After tracheal instillation of saline or acid, two week old rats were ventilated with $V_T$ 7 mL/kg and PEEP 5 cmH$_2$O or $V_T$ 21 mL/kg and PEEP 1 cmH$_2$O for 4 h. Airway resistance and the coefficient of tissue elastance, measured via low-frequency forced-oscillation technique, and quasi-static pressure-volume curves deteriorated less with high $V_T$-low PEEP when compared with low $V_T$-high PEEP. IL-6 concentration in bronchoalveolar lavage fluid (BALF) did not differ between all ventilated groups. Moreover, differences in BALF protein concentration and histological lung injury scores were independent of applied ventilation strategies. In contrast to both our hypothesis and experimental studies with adult rats, short-term mechanical ventilation with high $V_T$-low PEEP is not deleterious when compared to low $V_T$-high PEEP in healthy and pre-injured infant rat lungs. Our results call for caution when extrapolating data from adult studies and highlight the need for age-specific animal models.

Keywords

Tidal volume; positive end-expiratory pressure; ventilator-induced lung injury; mechanical ventilation; respiratory system mechanics; forced oscillation technique
1. Introduction

Almost 4 decades ago Webb and Tierney (1974) showed that volutrauma during mechanical ventilation with large tidal volume ($V_T$) induced acute lung injury (ALI) in adult rats with healthy lungs. Further experimental studies demonstrated that additional mechanisms such as repetitive opening and closing of peripheral lung units, shear forces, and inflammation caused by mechanical stressors were involved in the development of ventilator-induced lung injury (VILI) in animal models (Dreyfuss and Saumon, 1998; Slutsky, 1999).

Clinical studies avoiding high $V_T$ ventilation, low positive end-expiratory pressure (PEEP), and high peak inspiratory pressure (PIP) resulted in reduced mortality, improved lung function, and more ventilator-free days in adult humans (Hickling et al., 1990; Amato et al., 1995; ARDS network, 2000). Hence, confirmation of pre-clinical results led to implementation of lung protective ventilation strategies using low $V_T$, high PEEP, limited PIP, and tolerance towards moderate hypercapnia in clinical practice.

Based on clinical adult studies protective ventilation strategies were also recommended for infants and children with respiratory failure (Mehta and Arnold, 2004; Dahlem et al., 2007; Mesiano and Davis, 2008). Adoption of this concept is mainly due to a lack of both age-specific animal models and large clinical trials in the field of pediatrics (Khemani and Newth, 2010; Kneyber and Rimensberger, 2012) and does not take into account physiological and developmental age differences.

Results from the few infant animal models on VILI clearly demonstrated that younger rats with healthy lungs better tolerate high $V_T$ ventilation when compared to
adult rats (Copland et al., 2004; Kornecki et al., 2005). However, it is not known whether infant rats also tolerate a supposedly injurious high $V_T$-low PEEP ventilation strategy in the context of an ongoing inflammatory process. A key characteristic of adult VILI is that injurious ventilation more heavily affects pre-injured lungs of various etiologies when compared to healthy lungs (Frank et al., 2002; Quinn et al., 2002; Gurkan et al., 2003; Altemeier et al., 2004; Yang et al., 2008). Intratracheal acid instillation is often used to model ALI since it produces direct injury of airways and alveolar epithelium, patchy areas of neutrophilic inflammation, and impairment of lung function (Matute-Bello et al., 2008). Thus, the acid instillation model offers a possibility to investigate mechanical ventilation strategies in both healthy lungs and those with an ongoing inflammatory process.

The aim of this study was to test how so-called protective and injurious ventilation strategies affect lung function and inflammatory response in infant rats exposed to acid instillation. We hypothesised that acid-induced lung function impairment and inflammatory response are both aggravated by high $V_T$-low PEEP ventilation.
2. Methods

Experimental protocols were approved by the local Animal Experimentation Ethics Committee and were performed in accordance with Australian guidelines. Rat pups were kept under 12 h light and dark cycle and were housed with their parents and littermates. Piebald-Virol-Glaxo (PVG) rats used in this study are generally docile, have good health conditions, and show good breeding performance.

2.1 Acid instillation and animal preparation before mechanical ventilation

After inhalational anaesthesia with methoxyflurane 2 week old infant PVG rats (25.8 ± 1.5 g) were weighed and underwent oral intubation for intratracheal application of 75 µl saline or hydrochloric acid solution. Then, infant rats recovered in a warm environment and had to show adequate behaviour and activity before being returned to the parental cage.

Twenty-four h later, infant rats were weighed to both ensure normal food intake and to enter current body weight for \( V_T \) calculation in the \textit{flexiVent}\textsuperscript{®} system. Subsequently, infant rats were anaesthetised with an i.p. injection of a solution containing ketamine (80 µg/g) and xylazine (13 µg/g). A tracheostomy was performed and a 10 mm polyethylene cannula (ID: 0.86 mm) inserted. The rat was then placed in supine position on a heating mat and connected to a computer-controlled ventilator (\textit{flexiVent}\textsuperscript{®}, Scireq, Montreal, Canada) using the following settings: inspired oxygen fraction (FiO\(_2\)) 0.5, respiratory rate (RR) 90/min, \( V_T \) of 7 mL/kg, and PEEP 3 cmH\(_2\)O. Oxygen saturation
(SpO₂) was monitored via pulse oximeter (MouseOx™, STARR Life Sciences Corporation™, Oakmont PA, USA) by placing a sensor on the tail.

2.2 Respiratory system mechanics, pressure-volume curves, allocation to study groups

Lung volume history was standardized by application of 2 lung volume recruitment maneuvers with 40 mL/kg over 16 s within 2 min. A pressure-volume (PV) curve, consisting of a slow continuous ramp inflation from 3 to 20 cmH₂O and a deflation back over a total of 12 s, was then recorded to measure quasi-static compliance. Subsequently, baseline measurement of respiratory system input impedance (Z_{rs}) was performed using the low-frequency forced oscillation technique provided by flexiVent® system. Z_{rs} was obtained with a 4 s broadband signal between 1.0 and 20.5 Hz during a pause from mechanical ventilation. The “constant-phase” model, consisting of a single compartment comprising airway and tissue impedance elements connected in series, was fitted to the resulting Z_{rs} (Hantos et al., 1992), allowing the estimation of airway resistance (R_{aw}) and inertance, and the coefficients of tissue damping (G) and elastance (H). At each time point 4 respiratory system input impedance (Z_{rs}) spectra were collected within 120 s and the corresponding values were averaged. Inertance values got insignificantly low and hence are not reported. Since acid instillation leads to changes in airway resistance and lung compliance (Matute-Bello et al., 2008), we report R_{aw} and H, supposed to approximate airway resistance and lung elastance, respectively, to characterize alterations in lung function. Only after baseline measurements, rats (n=10 per group) were randomly allocated to one of the following ventilation strategies:
SLV and ALV: low $V_T$ ventilation with 7 mL/kg, PEEP of 5 cmH$_2$O, RR of 90/min, and FiO$_2$ of 0.5, following pre-treatment with saline or acid instillation the day before; SHV and AHV: high $V_T$ ventilation with 21 mL/kg, PEEP of 1 cmH$_2$O, RR of 30/min, and FiO$_2$ of 0.5, following pre-treatment with saline or acid. Animals were then ventilated for 4 h with $Z_{rs}$ measurements every 60 min. In order to avoid dehydration 0.3 mL of saline solution was given i.p. after the measurements at 1 and 3 h. Body temperature was maintained at 36.5-37.5°C with a heating pad.

After the last $Z_{rs}$ measurement at 4 h, ventilator settings were returned to baseline conditions, i.e. $V_T$ of 7 mL/kg, PEEP of 3 cmH$_2$O, and RR of 90/min in all animals to allow for comparison between PV curves obtained at baseline, after 4 h of ventilation, and after final recruitment. Hence, one min later, a second PV curve (“after 4 hours”) was performed. Subsequently, a third and final PV curve, preceded by 2 lung volume recruitment maneuvers with 40 mL/kg within 2 min (“following recruitment”), was applied before the animal was disconnected from the ventilator.

Except for mechanical ventilation, non-ventilated controls (SNV and ANV, n=5 per group) underwent the same procedures (pre-treatment, anesthesia, and tracheostomy) as the ventilated groups.

2.3 Sampling and processing of bronchoalveolar lavage fluid (BALF) and serum

Direct cardiac puncture at the end of the protocol was carried out under general anaesthesia and monitoring of SpO$_2$ and heart rate. Blood samples were allowed to clot before centrifugation. Serum was frozen for later analysis of macrophage inflammatory protein-2 (MIP-2), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). BALF was
collected from each animal by lavaging the lungs 3 times with 1.0 mL saline through the endotracheal tube. The supernatant was stored at -80°C until the measurement of IL-6, MIP-2, and TNF-α using ELISA kits (BD Biosciences, San Diego, California). Total protein was analysed using a colorimetric protein assay (Bio-Rad, Regents Park, NSW, Australia).

2.4 Morphologic analysis of lung tissues
Lungs were fixed by buffered 10% formalin instillation via the endotracheal tube at a pressure of 10 cmH₂O and then embedded in paraffin with the caudoventral aspect down. Sections were cut at 5-µm and stained with haematoxylin-eosin for light microscopy. A pathologist (PN) assessed slides (n=6 per group) in a blinded manner. Inflammation was assessed by first counting the number of inflamed foci as judged from a low power scan of the entire lung section. For each focus, the intensity of alveolar neutrophil and macrophage inflammation was scored on an arbitrary scale from 0 to 3. An assessment of the total inflammatory burden was then made by multiplying the number of inflamed foci by the intensity of alveolar neutrophil and macrophage inflammation at each focus, and calculating the mean for each group.

2.5 Statistical analysis
For group comparisons of SpO₂, peak airway opening pressure (Pₚₒ), Rₚₑ, H, PV curves, and cytokines and protein concentrations two-way ANOVA (factor 1: pre-treatment strategy, i.e. saline and acid; factor 2: ventilation strategy, i.e. low and high tidal) with Holm-Sidak post-hoc tests were used. One-way repeated measures ANOVA with Holm-
Sidak post-hoc tests were employed to compare $P_{ao}$ and $Z_{rs}$ changes over time within groups. One-way ANOVA with Holm-Sidak post-hoc tests were used to compare histological scores of acid-treated groups. Data were transformed (nat log, square root, reciprocal) where appropriate to ensure the assumptions of normality and equal variance were satisfied. Where this was not possible equivalent non-parametric comparisons were used (Kruskal-Wallis ANOVA on ranks). $P < 0.05$ was considered statistically significant.
3. Results

3.1 Airway resistance ($R_{aw}$) and lung tissue elastance ($H$)

At baseline, animals that had received hydrochloric acid solution showed small but significant higher $R_{aw}$ values (ALV 0.25; AHV 0.25 cmH$_2$O.s/mL) when compared with saline control groups (SLV 0.23; SHV 0.23 cmH$_2$O.s/mL) ($p=0.002$) (Fig. 1A). During ventilation $R_{aw}$ increased in all study groups when compared to baseline values. At the end of the study, based on the following $R_{aw}$ values for SLV 0.27, SHV 0.26, ALV 0.30, and AHV 0.28 cmH$_2$O.s/mL, we found small but significant differences between ventilation strategy (low vs. high $V_T$, $p=0.034$) and pre-treatment strategy (saline vs. acid, $p=0.002$).

Prior treatment with acid also resulted in significant higher $H$ values at baseline (SLV 17.8, SHV 17.9, ALV 19.6, AHV 19.8 cmH$_2$O/mL) ($p<0.001$) (Fig. 1B). Low $V_T$ ventilation resulted in a linear increase in $H$ by $\sim$100%, while high $V_T$ ventilation produced an initial increase in $H$ that levelled off over time. Thus, at the end of the protocol $H$ was 30% higher in the low $V_T$ groups than the high $V_T$ groups (SLV 40.3, SHV 31.2, ALV 43.0, AHV 32.5 cmH$_2$O/mL). Application of acid did not affect outcome values of $H$ ($p=0.12$).

3.2 Pressure-volume (PV) curves

At baseline acid exposed animals showed significantly lower volumes (SLV 0.6 mL, SHV 0.6 mL, ALV 0.54, AHV 0.53 mL) when reaching a $P_{aw}$ of 20 cmH$_2$O ($p=0.015$) (Fig. 2A). After 4 h of ventilation, we found a significant deterioration of lung
compliance in all animals (SLV 0.38 mL, SHV 0.54 mL, ALV 0.34, AHV 0.49 mL), though worsening was more pronounced for low \( V_T \) ventilation (\( p<0.001 \)) and acid exposure (\( p=0.008 \)) (Fig. 2B). The subsequent lung volume recruitment maneuver significantly increased volumes at \( P_{ao} \) of 20 cmH\(_2\)O in all groups (SLV 0.6 mL, SHV 0.67 mL, ALV 0.55, AHV 0.61 mL), but again resulting in significant differences between strategies (low vs. high \( V_T \), \( p<0.001 \)) and treatment (saline vs. acid, \( p=0.005 \)) (Fig. 2C). Intragroup comparison did not reveal significant differences between baseline and last PV curves in both low \( V_T \) groups (\( p=0.96 \) and \( p=0.26 \)). In contrast, high \( V_T \) ventilation resulted in significantly better lung compliance after the last recruitment maneuver when compared to baseline PV curves.

3.3 Peak airway opening pressure (\( P_{ao} \)) and oxygen saturation (\( \text{SpO}_2 \))

Peak \( P_{ao} \) values were only recorded at baseline, after allocation to low \( V_T \)-high PEEP or high \( V_T \)-low PEEP strategy, and at the end of the study before the last lung volume recruitment maneuver. In all animals \( \text{SpO}_2 \) was monitored during baseline measurements and at the end of the study to ensure that animals were alive. Random controls of \( \text{SpO}_2 \) were not taken at predefined time points. Peak \( P_{ao} \) and \( \text{SpO}_2 \) values from the selected time points are presented in Table 1. Overall, pre-treatment with acid did not result in significant higher peak \( P_{ao} \) and \( \text{SpO}_2 \) values at baseline (\( p>0.10 \) in all cases).

3.4 Total protein and cytokine concentrations in BALF
Serum MIP-2, IL-6, and TNF-α concentrations as well as BALF MIP-2 and TNF-α concentrations were below detection levels. Thus, only total protein and IL-6 concentrations in BALF are illustrated in Table 1.

3.5 Lung morphology

The total inflammatory burden, i.e. number of inflamed foci multiplied by the neutrophil and macrophage scores, was significantly greater in animals exposed to acid instillation (Table 1). Ventilation strategy did not affect inflammatory burden in acid-treated infant rats (p=0.49 for neutrophils and p=0.80 for macrophages). Characteristic photomicrographs are presented in Figure 3.
4. Discussion

To investigate effects of protective and injurious ventilation strategies on respiratory system mechanics and inflammatory response in infant rats we used an acid injury model. Acid-induced lung injury only partially reproduces features of pediatric ALI following viral or bacterial infection, sepsis, or trauma. Nonetheless, acid injury models are useful since they induce histopathological changes such as inflammation, alveolar and interstitial edema, hemorrhage, and necrosis resulting in increased airway resistance and decreased lung compliance (Matute-Bello et al., 2008). Hence, this model offers an opportunity to study some of the pathologies found in pediatric ALI. The major findings of the present study were as follows: (1) high VT-low PEEP ventilation did not aggravate lung injury in infant rats with pre-injured lungs, (2) mechanical ventilation per se led to increased IL-6 concentrations in BALF, and (3) changes in lung function were completely reversed by a lung volume recruitment maneuver.

In terms of lung development and alveolarization 2 week old infant rats used in this study can be compared to 1-2 year old human infants (Thurlbeck, 1982; Burri, 2006; Bolle et al., 2008), hence, providing a useful model to investigate pediatric lung injury associated with mechanical ventilation. Compared to controls acid instillation produced interstitial and alveolar inflammation and high BALF protein concentrations, which was matched by a significant increase in $R_{aw}$ and $H$ and a significant decrease in quasi-static compliance of the respiratory system at baseline.

The pattern of changes of respiratory system mechanics over time was clearly affected by the ventilation strategy. Both dynamic and quasi-static measurements revealed more pronounced deterioration of lung function in animals receiving low $V_T$ and
high PEEP ventilation. The linear increase in H most likely reflects a progressive loss of lung volume despite PEEP (Irvin and Bates, 2003; Allen et al., 2007; Cannizzaro et al., 2008). This assumption is also supported by the response to lung volume recruitment maneuvers at the end of the study. The small, but persistent difference in $R_{aw}$, H, and quasi-static lung compliance between saline and acid exposed animals suggests that alterations in lung mechanics were mainly due to volume changes and only to a lesser extent to structural changes related to inflammation and increased epithelial permeability. Complete lung volume recruitability emphasises the potential and importance of recruitment maneuvers during low $V_T$ ventilation.

Kornecki and et al. (2005) measured quasi-static lung compliance in healthy infant and adult rats ventilated with 20 cmH$_2$O hereby resulting in a $V_T$ of $>37$ mL/kg and $>18$ mL/kg, respectively, and at 1 cmH$_2$O PEEP. No changes in total lung compliance were found in infants after 90 min, whereas adult rats showed a significant deterioration in lung compliance. During the first 60 min of our study, high $V_T$-low PEEP ventilation led to a similar steep increase in H as in the low $V_T$-high PEEP groups. However after that, the rise in H stopped and remained significantly lower than H in animals receiving low $V_T$-high PEEP ventilation. Similarly, compliance obtained from PV curves decreased when compared to baseline, but was significantly better then after the low $V_T$-high PEEP strategy. We guess that application of high $V_T$ resulting in higher peak $P_{ao}$ of ~15.0-20.7 cmH$_2$O (compared to ~10.5-13.7 cmH$_2$O in the low $V_T$ groups) prevented further increase in H via alveolar recruitment. The moderate decrease in compliance, assessed via H and PV curves, without additional signs of lung injury
provides further evidence that the high V_T-low PEEP strategy does not exacerbate acid-induced lung injury in infant rats.

Based on results from adult rat studies (Dreyfuss and Saumon, 1998; Al-Jamal and Ludwig, 2001; Pavone et al., 2007) it was suggested that high V_T ventilation, particularly in combination with low PEEP, reduces aerated lung volume causing regional heterogeneity and produces alveolar instability via surfactant deactivation. In addition, it was hypothesised that redistribution of ventilation from non-aerated to aerated lung units results in overinflation injury (Tsuchida et al., 2006). In line with the concepts of overinflation and alveolar instability from regional heterogeneity and surfactant deactivation, respectively, it was found that low V_T as opposed to high V_T ventilation attenuated acid-induced lung injury in adult rats (Frank et al., 2002; Gurkan et al., 2003). In our study, application of 5 cmH_2O PEEP did not prevent substantial loss of lung volume and resulted in worsened lung function. While high V_T ventilation in infant rats with very compliant chest walls (Bolle et al., 2008; Gomes et al., 2001) had the potential to prevent derecruitment, peak P_ao and mechanical forces generated during low V_T ventilation were probably insufficient to reopen atelectatic lung units in our study. As expected application of recruitment maneuvers rapidly improved lung compliance in all animals. Nonetheless, it is remarkable that a significant difference in lung compliance between low and high V_T groups persisted. This finding challenges the view that surfactant dysfunction, as a proposed VILI mechanism in adult rodent studies (Allen et al., 2007; Pavone et al., 2007; Chiumello et al., 2008) plays a major role in infant rats with both healthy and pre-injured lungs receiving short-term high V_T ventilation.
We also measured protein and cytokine concentrations in BALF as well as lung morphological alterations as surrogates of lung injury and inflammation. The difference in protein levels was clearly linked to acid instillation. The elevated IL-6 concentrations in all ventilated groups either imply that ventilation protocols were too short to cause significant differences in biotrauma (Dos Santos and Slutsky, 2006; Bailey et al., 2008) or that a similar degree of mechanical stress occurred during artificial ventilation with both strategies. Overall, our results from both inflammatory response and lung mechanics are consistent with a previous infant rat study showing improvement in lung mechanics following high \( V_T \)-low PEEP when compared with low \( V_T \)-high PEEP ventilation in an indirect lung injury model (Cannizzaro et al., 2010). Despite these experimental findings it is too early to draw direct conclusions for clinical practice. More experimental research with age-specific animal models and longer protocols with less extreme ventilation strategies will be needed.

The following limitations need to be acknowledged. First, the 4 h duration of our mechanical ventilation protocol limits extrapolation to clinical practice. However, it has to be considered that conclusions drawn from the majority of previous studies performed in the context of ventilator-induced lung injury (VILI) in infant and adult rodents were based on shorter protocols. Longer protocols in future experimental VILI studies should be strived for in order to increase translational value. Second, we did not measure blood gas values, hence it remains unclear whether hypercapnic acidosis occurred in one of the two ventilation strategies. This is a potential bias since hypercapnic acidosis has been shown to influence and decrease severity of VILI (Halbertsma et al., 2008). Third, acid-induced lung injury only partially mimics clinical ALI in infants and children.
Nonetheless, the acid instillation model is characterized by neutrophil infiltration, edema, atelectasis, inflammation, and worsening of lung mechanics (Henzler et al., 2011). In fact, these features can also be encountered in critically ill children with ALI. Lastly, the high V_T-low PEEP strategy used in this study is clearly not recommended in human infants and does not reflect management in clinical practice.

In conclusion, we showed that application of high V_T-low PEEP is superior to low V_T-high PEEP in terms of lung tissue elastance and quasi-static pressure-volume curves after short-term mechanical ventilation in infant rats. Furthermore, in contrast to comparable adult rodent studies, we found no evidence that high V_T-low PEEP ventilation exacerbates pre-existing lung injury induced by acid instillation. The presented results call for age-specific animal models and caution when adopting therapeutic concepts derived from adult studies.

5. Disclosure
The authors declare that they have no conflict of interests.

6. Acknowledgements
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7. References


8. Figure legends

Figure 1
Airway resistance $R_{aw}$ (panel A) and lung tissue elastance $H$ (panel B) as a function of time on the ventilator. Data are presented as group means ± standard error of the mean (distribution of 4 averaged $R_{aw}$ and $H$ values for each time point and infant rat). “*” denotes significant differences between saline and acid groups at baseline; over time $R_{aw}$ and $H$ significantly increased in all study groups when compared with baseline values; “+” shows significant differences related to both pre-treatment and ventilation strategy at the end of the study; “^” displays significant differences related to ventilation strategy only at the end of the study.

Figure 2
Pressure-volume curves produced via continuous slow inflation-deflation from 3 to 20 cmH$_2$O and back. Panels A, B, and C show changes in airway opening pressure ($P_{ao}$) plotted against volume at baseline before allocation to ventilation strategies, after 4 h of ventilation following return to baseline conditions, and after a lung volume recruitment maneuver, respectively. Please note that the 2 saline and 2 acid-treated groups overlap at baseline, hence giving the erroneous impression of 2 groups only. Data are expressed as group means (± standard deviation for $P_{ao}$ at 20 cmH$_2$O only). Significant differences in volume at a $P_{ao}$ of 20 cmH$_2$O were related to pre-treatment strategy “*” (saline versus acid) or both pre-treatment and ventilation strategy “+”.
Figure 3

Illustrative photomicrographs of representative lung fields. A: area of normal lung with no/few neutrophils and macrophages. B and C: alveolar wall thickening and numerous neutrophils and macrophages. D: moderate alveolar oedema, shown as faint pink-staining fluid material within the airspace. E: frequent alveolar macrophages. F: frequent alveolar neutrophils
### Table

**Table 1** Peak airway opening pressure (P<sub>ao</sub> in cmH<sub>2</sub>O), oxygen saturation (SpO<sub>2</sub> in %) at selected time points, BALF protein (mg/mL) and interleukin-6 (pg/mL) concentrations, and inflammatory burden of neutrophils and macrophages

<table>
<thead>
<tr>
<th></th>
<th>Peak P&lt;sub&gt;ao&lt;/sub&gt; at baseline</th>
<th>Peak P&lt;sub&gt;ao&lt;/sub&gt; after allocation to low or high VT</th>
<th>SpO&lt;sub&gt;2&lt;/sub&gt; at baseline</th>
<th>SpO&lt;sub&gt;2&lt;/sub&gt; at study end</th>
<th>BALF protein concentration</th>
<th>BALF interleukin-6 concentration</th>
<th>Inflammatory burden of neutrophils</th>
<th>Inflammatory burden of macrophages</th>
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<td>SNV</td>
<td>0.15 (0.04)</td>
<td>b.d.</td>
<td>0 (0)</td>
<td>0.15 (0.4)</td>
<td>5.2 (3.3)</td>
<td>4.8 (3.3)</td>
<td>0 (0)</td>
<td>0.2 (0.4)</td>
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<tr>
<td>ANV</td>
<td>1.17 (0.4)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>b.d.</td>
<td>5.2 (3.3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.74 (0.3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5.2 (3.3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.8 (3.3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>3.2 (2.9)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.5 (4.1)&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>SLV</td>
<td>7.6 (0.2)</td>
<td>10.5 (0.7)</td>
<td>97.9 (0.8)</td>
<td>97.8 (2.2)</td>
<td>0.15 (0.03)</td>
<td>406 (91)</td>
<td>0 (0)</td>
<td>0.2 (0.4)</td>
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<td>ALV</td>
<td>7.8 (0.3)</td>
<td>11.2 (1.0)</td>
<td>95.6 (2.8)</td>
<td>94.0 (2.5)</td>
<td>0.74 (0.3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>280 (174)</td>
<td>3.2 (2.9)&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>SHV</td>
<td>7.6 (0.3)</td>
<td>15.0 (2.2)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>97.9 (0.4)</td>
<td>99.1 (0.8)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.20 (0.03)</td>
<td>522 (146)</td>
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<td>AHV</td>
<td>8.0 (0.5)</td>
<td>18.1 (4.8)&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>3.5 (2.5)&lt;sup&gt;+&lt;/sup&gt;</td>
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Data are expressed as group means ± SD. * indicates a significant difference when compared to low VT groups. # indicates a significant increase when compared to baseline value. + denotes a significant difference between saline and acid-treated animals with no statistical difference within the latter group. SNV, saline non-ventilated; ANV, acid non-ventilated; SLV, saline low VT; ALV, acid low VT; SHV, saline high VT; AHV, acid high VT; “b. d.” denotes concentration of cytokine below detection limit of the assay.