The effects of tidal volume size and driving pressure levels on pulmonary complement activation: an observational study in critically ill patients

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Abstract

Background: Mechanical ventilation can induce or even worsen lung injury, at least in part via overdistension caused by too large volumes or too high pressures. The complement system has been suggested to play a causative role in ventilator-induced lung injury.

Aims and methods: This was a single-center prospective study investigating associations between pulmonary levels of complement activation products and two ventilator settings, tidal volume (VT) and driving pressure (ΔP), in critically ill patients under invasive ventilation. A miniature bronchoalveolar lavage (BAL) was performed for determination of pulmonary levels of C5a, C3b/c, and C4b/c. The primary endpoint was the correlation between BAL fluid (BALF) levels of C5a and VT and ΔP. Levels of complement activation products were also compared between patients with and without ARDS or with and without pneumonia.

Results: Seventy-two patients were included. Median time from start of invasive ventilation till BAL was 27 [19 to 34] hours. Median VT and ΔP before BAL were 6.7 [IQR 6.1 to 7.6] ml/kg predicted bodyweight (PBW) and 15 [IQR 11 to 18] cm H₂O, respectively. BALF levels of C5a, C3b/c and C4b/c were neither different between patients with or without ARDS, nor between patients with or without pneumonia. BALF levels of C5a, and also C3b/c and C4b/c, did not correlate with VT and ΔP. Median BALF levels of C5a, C3b/c, and C4b/c, and the effects of VT and ΔP on those levels, were not different between patients with or without ARDS, and in patients with or without pneumonia.

Conclusion: In this cohort of critically ill patients under invasive ventilation, pulmonary levels of complement activation products were independent of the size of VT and the level of ΔP. The associations were not different for patients with ARDS or with...
Background pneumonia. Pulmonary complement activation does not seem to play a major role in VILI, and not even in lung injury per se, in critically ill patients under invasive ventilation.

Keywords: Intensive care, Critical care, Mechanical ventilation, Tidal volume, Driving pressure, Bronchoalveolar lavage, Complement, Complement activation, Complement component 5, C5a

Background

Invasive ventilation has a strong potential to cause so-called ventilator-induced lung injury (VILI) [1], at least in part via overdistension of lung units due to the use of too large volumes or too high pressures [2]. Ventilation with a low tidal volume ($V_T$) of 6 ml/kg predicted body weight (PBW) clearly improves outcomes in patients with acute respiratory distress syndrome (ARDS) [3], and maybe also in patients without ARDS [4, 5]. Ventilation with a high driving pressure ($\Delta P$), the difference between plateau pressure and positive end-expiratory pressure (PEEP), has been associated with worse outcomes in patients with ARDS [6, 7], and a cut-off value of 15 cm H$_2$O for $\Delta P$ has been proposed and is currently widely used at the bedside as a safety limit.

The pathophysiological mechanisms of VILI remain only partly understood. Complement activation has been suggested as one pathogenetic factor in VILI [8]. However, evidence for a key role of complement activation persists to be poor and originates mainly from preclinical studies. In rats with Streptococcus pneumoniae pneumonia, ventilation with a high $V_T$ increased pulmonary levels of the complement activation product C4b/c [9]. In healthy mice, ventilation with a high $V_T$ resulted in increased complement C3 deposition in the lung and reduced cell aggregation [10]. In healthy rats, ventilation with a high $V_T$ increased pulmonary vascular permeability, a finding that was linked to increased plasma levels of soluble terminal complement complex [11]. Also, in a study with healthy rats, ventilation with high airway pressures resulted in increased C3a levels in plasma [12]. Studies in the human setting are even more scarce, and findings in these studies are conflicting with those in preclinical studies. For instance, no association was found between complement depositions in lung tissue and $\Delta P$ in critically ill patients who died under invasive ventilation [13].

We initiated the current study to gain a better understanding of the effects of $V_T$ and $\Delta P$ on complement activation in the pulmonary compartment of critically ill patients under invasive ventilation. The hypothesis was that pulmonary complement activation is associated with $V_T$ and $\Delta P$, and also that pulmonary levels of complement activation products are higher in patients with ARDS or with pneumonia.

Methods

Study design and ethical concerns

This was a sub-study of the prospective observational ‘Biomarker Analysis in Septic Intensive Care patients’ (BASIC) study. BASIC was a single-center investigation performed in the intensive care unit (ICU) of the Amsterdam University Medical Centers, location ‘AMC’, Amsterdam, The Netherlands. The study protocol of BASIC was approved by the Institutional Review Board (METC 2010_335#B201112). The study was registered at the Dutch Central Commission for Human bound Research (CCMO)
Patients

Patients were eligible for participation in BASIC if: (a) aged 18 years or older; (b) expected ICU stay of at least 24 h; and (c) having at least two criteria for systemic inflammatory response syndrome (SIRS) [14], with or without infectious causality. Readmitted patients, patients who were referred to the participating ICU from an ICU in another hospital, patients treated with antibiotics for >48 h, patients included in other studies testing interventions that could possibly affect inflammatory processes, and patients of whom no written informed consent was obtained were excluded. For the current analysis, we included patients who were under invasive ventilation and were subjectable to a bronchoalveolar lavage (BAL) (see below). Patients were also excluded if there was no blood sample taken at the moment of the BAL, or if BAL fluid (BALF) was of insufficient quality (see below for definition).

Clinical data and ventilator variables and parameters

A dedicated team of trained researchers collected baseline characteristics and outcomes, and scored presence of ARDS according to the Berlin definition for ARDS [15]. Presence of pneumonia was assessed using the Centers for Disease Control and Prevention and International Sepsis Forum consensus definitions [16]. Patients with pneumonia on admission and patients having pneumonia within 48 h of start of ventilation, were considered pneumonia patients.

Granular ventilation data were collected from the electronic patient data monitoring system that recorded and stored (a) ventilation mode, (b) expired \( V_T \), (c) PEEP and (d) maximum, peak and plateau airway pressures, and (e) fraction of inspired oxygen (FiO\(_2\)) every 5 min.

BAL

Within 48 h after ICU admission a miniature BAL was performed as described before [17]. In short, a 50-cm 14-gauge tracheal suction catheter was inserted via the orotracheal tube and advanced until significant resistance was encountered. Then 20 ml of sterile normal saline was instilled over a period of 4 to 5 s. Immediately hereafter, fluid was aspirated, typically recovering 4 to 8 ml. This BALF was processed directly and centrifuged at 1.500 \( \times \) g for 15 min at 4 °C; samples were stored at −80 °C until assays were performed batchwise.

Assays

The following complement activation products were measured in BALF to determine complement activation. C5a was measured using a commercial enzyme-linked immunosorbent assays (MicroVue, Quidel, San Diego, CA). C3b/c and C4b/c were measured using home-made enzyme-linked immunosorbent assays (Sanquin, Amsterdam, The Netherlands) as described before [18, 19]. As these assays do not distinguish,
respectively, C3b from C3bi and C3c, and C4b from C4bi and C4c, we referred to these as C3b/c and C4b/c.

Urea levels were determined in BALF and plasma using quantitative colorimetric urea determination (BioAssay Systems, Hayward, CA).

**Primary and secondary endpoints**

The coprimary endpoint was the association between complement activation product C5a in BALF and median $V_T$ and median $\Delta P$ from start of invasive ventilation till the BAL.

Other endpoints were the association between pulmonary levels of C5a and median $V_T$ and $\Delta P$ in the 6-h time-frame before BAL, associations between other complement activation products and these two ventilator settings, and local levels of C5a in patients with ARDS versus patients without ARDS, and in patients with pneumonia versus patients without pneumonia.

**Power calculation**

Data on which we could base a power calculation were lacking. Therefore, we used samples from all patients who were included in the BASIC study, who were under invasive ventilation and underwent a BAL within 48 h after its initiation. With 72 analyzable patients, we have a two-sided significance level of 0.05 and a power of 80% for correlation coefficient ($r$) as low as 0.325.

**Analysis plan**

Continuous variables were presented as median (25th–75th interquartile range [IQR]) or mean with standard deviation (SD), where appropriate. Categorical variables are shown in proportions (%). Continuous variables were analyzed using a Mann–Whitney U-test or Student’s t-test according to data distribution, proportions were compared using a Fisher exact test. From the available 5-min ventilation data, first the median $V_t$, $\Delta P$ and PEEP level was calculated for each patient. Then these medians were used to calculate the median and IQR of the whole group. $V_T$ was expressed in ml/kg predicted bodyweight (PBW) [20]. $\Delta P$ was calculated by subtracting PEEP from the maximum airway pressure, as all patients were under pressure-controlled modes of ventilation and no plateau airway pressures were available [21, 22].

To correct BALF levels of C5a, C3b/c, and C4b/c for differences caused by dilution of the samples, the ratio between urea in BALF and urea in plasma was used to correct complement concentrations in BALF, as described before [23]. Patients with ‘low quality’ BALF, defined as samples with urea levels below the detection limit, were excluded from the analyses. Levels were presented for the whole group, and for patients with ARDS or pneumonia. Levels were individually plotted on a log-scale together with Tukey boxplots.

The association between levels of pulmonary complement activation and $V_T$ and $\Delta P$ was analyzed in two ways. First, the association with $V_T$ and $\Delta P$ from start of invasive ventilation till BAL was investigated. Also, the association with ventilation in the 6 h before BAL was investigated. We used scatterplots and $r$ using Pearson’s/Spearman association method according to data distribution. The complete analysis was repeated to
compare the effects of ventilator settings on complement activation products in patients with ARDS versus those without ARDS, and in patients with pneumonia and patients without pneumonia.

Statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad software Inc, La Jolla, CA, USA). A $P$-value of <0.05 was considered statistically significant.

**Results**

**Patients**

Flowchart of patients is shown in Fig. 1. In total, 355 patients were included in the BASIC study. After excluding patients who were not under invasive ventilation, patients who did not have a matched BALF to plasma sample, and patients in whom BALF was considered of poor quality, 72 patients remained for the current analysis. Of these patients, 21 patients (29%) were classified as having ARDS, and 29 patients (40%) had pneumonia. Patient characteristics are presented in Table 1. ICU mortality rate was 25%.

Median time from start of invasive ventilation to BAL was 27 [IQR 19 to 34] hours. Median $V_T$ from start of ventilation to BAL was 6.7 [IQR 6.1 to 7.6] ml/kg PBW, comparable to the median $V_T$ of 6.8 [IQR 6.1 to 8.0] ml/kg PBW in the last 6 h before the

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**Fig. 1** Flow of patients. ICU intensive care unit, SIRS systemic inflammatory response syndrome, BASIC-study ‘Biomarker Analysis in Septic Intensive Care patients’-study, BAL bronchoalveolar lavage, BALF bronchoalveolar lavage fluid
Median ΔP from start of ventilation to BAL was 15 [IQR 11 to 18] cmH\(_2\)O, slightly higher than the median ΔP of 13 [IQR 9 to 18] cmH\(_2\)O in the last 6 h before BAL. Median PEEP from start of ventilation to BAL was 7.8 [IQR 5.0 to 10.0] cmH\(_2\)O, higher than the median PEEP of 6.0 [IQR 5.0 to 10.0] cmH\(_2\)O in the last 6 h before BAL.

**BALF levels of complement activation products**

Median levels of complement activation products in BALF are shown in Fig. 2. Median C5a level was 103 [IQR 49–307] ng/ml, median C3b/c and C4b/c levels were 739 [IQR 471–1939] and 79 [IQR 38–179] nmol/L, respectively. Complement activation products in BALF were similar in patients with ARDS or without ARDS. The same was found for patients with or without pneumonia.

**Correlations between pulmonary complement activation and \(V_T\) and ΔP**

The correlation between BALF levels of complement activation product C5a and \(V_T\) and ΔP was poor, as illustrated in Fig. 3. Correlations were also poor between BALF levels of the other two complement components and median \(V_T\) and median ΔP.

Restricting the analysis to ventilation data collected within the last 6 h before the lavage, did not change the results (see Additional file 1: Figure S1). Comparing...
associations between $V_T$ and $\Delta P$ in patients with ARDS versus patients without ARDS, and in patients with or without pneumonia resulted in comparable findings (see Additional file 2: Figure S2 and Additional file 3: Figure S3).

**Discussion**

The results of this study in a cohort in critically ill patients under invasive ventilation for various reasons can be summarized as follows: (a) no association was found between pulmonary levels of complement activation products and two main ventilator settings, $V_T$ and $\Delta P$, and (b) this was neither different for patients with ARDS and patients without ARDS, nor for patients with pneumonia and patients without...
pneumonia; (c) one other salient finding was that pulmonary levels of complement activation products were similar between patients with and patients without ARDS or pneumonia.

This study knows several strengths. Bias was minimized by its prospective character, and a preplanned analysis plan was strictly followed. The clear inclusion and exclusion criteria led to a recognizable population of critically ill patients with high severity of illness scores. The number of included patients was large, exceeding the numbers of several preceding studies. Patients underwent a BAL relatively soon after start of invasive ventilation. Last but not least, the electronic patient data monitoring system allowed us to use granular ventilation data, providing an accurate reflection of ventilator settings before BAL in all patients.

Since complement factors are unstable, we payed extensive attention to proper handling of the BAL samples. Samples were immediately processed and stored. All analyses were performed batchwise. Therefore, an effect of complement instability on our results is very unlikely. With a miniature BAL, differences in dilution can result in erroneously low levels of biomarkers of interest, in this case complement activation products. Differences in urea concentration in BAL and plasma were used to correct for this inaccuracy [23]. We improved the quality of our data collection by removing samples from the final analysis when BALF urea levels suggested too much dilution.

Though no data exist on levels of complement activation products in BALF in humans with VILI, several studies have been performed in patients with ARDS [24–26]. One of these studies used C5a as the marker of complement activation, alike we used in the current investigation [24]. In that study, median levels of C5a in BALF were ~400 ng/ml, fourfold higher than the median level found in our study. It should be noted, though, that there were important differences in sampling (a miniature BAL in the current study vs a formal BAL in the previous one) as well as sample handling (no further processing in the current study versus concentrating supernatant using pressure filtration. Last but not least, the two investigations used different assays for C5a measurements.

The results of the current investigation are in contrast with findings in previous animal studies [9–12]. In rodent models of VILI, ventilation with a high VT [9–11] or a high ventilation pressure [12] resulted in clear complement activation, seen the increased levels of complement activation products. It must be mentioned, that in those studies VILI was induced by ventilation with a high VT (12 ml/kg) [9] to an extreme high VT (35 ml/kg [10] or 40 ml/kg [11]), or with ventilation with a high peak pressure, and thus probably a high ΔP [12]. Those settings no longer reflect current clinical practice. Indeed, in the current study, VT and ΔP were all within widely recommended ranges [27, 28].

One other important difference between preceding animal studies and the current human investigation is that in the animal studies identical hits could be used in a well-controlled setting in genetically comparable rodents, while the cohort of patients in the current study was heterogeneous and had a variety of pulmonary hits. But even with use of a relatively large cohort of patients, to correct for these variances, no correlation was found between pulmonary levels of complement activation products and the two ventilator settings of interest.

The findings of the current study are in line with one previous human study of our group. In a series of critically ill patients who died under invasive ventilation, we found
no association between complement C3d depositions in lung tissue and $\Delta P$ in the final hours before death [13]. No difference in deposition of C3d in ARDS patients when compared to patients without ARDS was present. Two other studies showed increased BALF levels of complement activation products in ARDS patients [24, 25]. It must be mentioned that in these studies complement levels in patients were compared to levels in healthy volunteers and postoperative patients, and not to levels in critically ill patients without ARDS. In one other study, a difference in levels of pulmonary complement activation was found between trauma patients who developed ARDS and those who did not, but these differences were only present very early after start of ventilation, i.e., within hours after its initiation [26]. It is possible that we were ‘too late’ to find a difference in complement activation in the current study.

Several limitations need to be mentioned. With the use of medians to reflect $V_T$ and $\Delta P$ during invasive ventilation, shorter periods of more injurious ventilator settings could have been missed. As mentioned above, this also means that we cannot exclude a possible effect of (much) higher $V_T$ and $\Delta P$ on pulmonary complement activation. Use of miniature BAL might be inferior to a formal BAL. However, previous studies showed no differences between these two techniques with regard to, e.g., counts of colony forming units [17]. Miniature BAL has the advantage of being less invasive and is therefore frequently used in studies as alternative to a formal BAL [29–31]. Finally, complement activation within the pulmonary compartment in critically ill patients is possibly already high at baseline. This may partially mask the effects on complement activation of any ventilator setting.

**Conclusion**

In this cohort of critically ill patients under invasive ventilation for various reasons, no association between levels of pulmonary complement activation and $V_T$ or $\Delta P$ was found. It could be that pulmonary complement activation does not play a major role in VILI, and not even in lung injury per se. If true, treatment with complement inhibitors may not contribute to a better outcome in critically ill patients under invasive ventilation.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s40635-020-00356-6.

Additional file 1: Figure S1. Association between complement activation products, C5a (A + D), C3b/c (B + E) and C4b/c (C + F) in bronchoalveolar lavage fluid and tidal volume (A-C) and driving pressure (D-F) in the last 6 h before BAL. Abbreviations: C, complement activation product; $V_T$, tidal volume; $\Delta P$, driving pressure.

Additional file 2: Figure S2. Association between complement activation products C5a (A + D), C3b/c (B + E) and C4b/c (C + F) in bronchoalveolar lavage fluid and tidal volume (A-C) and driving pressure (D-F) in patients with (closed symbols) and patients without acute respiratory distress syndrome (open symbols). Abbreviations: C, complement activation product; $V_T$, tidal volume; $\Delta P$, driving pressure.

Additional file 3: Figure S3. Association between complement activation products C5a (A + D), C3b/c (B + E) and C4b/c (C + F) in bronchoalveolar lavage fluid and tidal volume (A-C) and driving pressure (D-F) in patients with (closed symbols) and patients without pneumonia (open symbols). Abbreviations: C, complement activation product; $V_T$, tidal volume; $\Delta P$, driving pressure.

**Abbreviations**

ARDS: Acute respiratory distress syndrome; BAL: Bronchoalveolar lavage; BALF: Bronchoalveolar lavage fluid; ICU: Intensive care unit; BMI: Body mass index; PBW: Predicted bodyweight; APACHE: Acute Physiology and Chronic Health Evaluation; SAPS: Simplified Acute Physiology Scores; FiO$_2$: Fraction of inspired oxygen; PEEP: Positive end-expiratory pressure;
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Authors' contributions
FB, TP, NJ, JH, MS and WL conceived the study. FB, LW and LB performed bronchoalveolar lavages and obtained blood samples. DW and SZ provided antibodies and facility for complement measurements. FB and GM performed the complement measurements. FB and LW analyzed and interpreted the data. FB, JH, MS and WL drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study protocol of BASIC was approved by the Institutional Review Board (METC 2010_335#B201112). Written informed consent was obtained from all patients or their next of kin, before study entry.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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SIRS: Systemic inflammatory response; VILI: Ventilator-induced lung injury; V_t: Tidal volume; ΔP: Driving pressure; IQR: Interquartile range; SOFA: Sequential Organ Failure Assessment; COPD: Chronic obstructive pulmonary disease.
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