Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets

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Abstract

Background: Many successful therapies developed for human medicine involve animal experimentation. With competition for public research funding and career advancement opportunities, animal studies focused on the translational potential may not sufficiently document unexpected outcomes. Such studies often have hastily developed methods with ad hoc modifications including the use of additional animals, leading to considerable amounts of idle, unprocessed data that could be used to advance veterinary science, or to refine the base animal model. Sheep, for example, are poorly understood models of intensive care and therefore, any experimental data arising from them should be interpreted with care. The hypothesis was that there is little information describing the development of methods of physiological data processing in multifaceted sheep models of intensive care and the author aimed to develop a suitable data processing method and to analyse the data, once processed.

Methods: Data from 19 adult mechanically ventilated ewes undergoing intensive care in a previous study evaluating a form of extracorporeal life support (treatment) for acute lung injury were used to develop a comprehensive method for processing manual and electronically gathered clinical observations. Eight sheep were injured by acute smoke inhalation prior to treatment (injured/treated), while another eight were not injured but treated (uninjured/treated). Two sheep were injured but not treated (injured/untreated), while one received room air instead of smoke as the injury, and was not treated (placebo/untreated). The data were then analysed for 11 physiological categories and compared between the two treated groups.

Results: The analysis revealed that compared with the baseline, treatment contributed to and exacerbated the deterioration of pulmonary pathology by reducing lung compliance and PaO$_2$/FiO$_2$ ratio. The oxygen extraction index changes mirrored those of the PaO$_2$/FiO$_2$ ratio. Decreasing coronary perfusion pressure predicted the severity of cardiopulmonary injury.

Conclusions: These novel observations could help in understanding similar pathology such as that which occurs in smoke inhalation animal victims of house and bush fires. A similar data processing method could be used when evaluating the effectiveness of other clinical interventions such as potentially reversible aspiration pneumonia secondary to tick paralysis in veterinary patients.

Keywords: Sheep, critical care, smoke-induced acute smoke inhalation injury, extra-corporeal life support, lung compliance, PaO$_2$/FiO$_2$ ratio
During multifaceted experiments involving intensive care in large animal models in translational research, information regarding animal monitoring is often collected with varying accuracy, scope, and end-user applications. Data collection can be manual, electronic, or both [1-3]. Manually input data can include subjectively scored end-points such as the plane of anaesthesia, and objective data such as heart rate or breaths per minute. Depending on the goals of the study, some information may be used to validate or test novel therapies, or to understand and refine existing treatments. In some cases, experimental information may be gathered for scientific curiosity or for “classified” use, and outcomes may never be publicly available, especially if the results are negative.

The source of data for this study was from a sheep model [2-4] being treated for smoke-induced acute lung injury using veno-venous extracorporeal membrane oxygenation [2], a form of extracorporeal life support (ECLS) developed to complement the treatment of acute lung injury in humans [5-7]. During this type of ECLS, venous blood is carried from the patient to a gas exchange device where it becomes enriched with oxygen, has carbon dioxide removed, and is returned to the patient’s circulation in the right heart. This method can be used for treatment, as respiratory support during lung transplantation, and in critically ill patients with potentially reversible respiratory failure [7]. The multiple advanced cardiovascular [3], respiratory, patient point-of-care procedures, and instrumentation associated with ECLS, even in animal experimentation, is highly data- and equipment-intensive. This platform is useful for developing research and methodological skills for in vivo animal instrumentation and the processing of large, real-time clinical data sets from multifaceted animal studies that can be applied to similar intensive care scenarios. An opportunity to develop these skills arose within the source study, which was an ongoing publicly funded animal experimentation study (Queensland University of Technology Animal Ethics Approval No. 110000053). While the objectives of the primary study had a separate focus, there were considerable amounts of redundant raw data with potential use in veterinary science and other disciplines, once processed. The author hypothesised that there was little information describing the development of methods of physiological data processing in multifaceted large animal models of intensive care.

The overall goal was to provide useful information relevant to the sheep model, itself, and to those interested in large animal experimentation and veterinary medicine, generally. The specific objectives were: 1) use the raw data from the sheep model study to create a data management system for tabulating large data sets from human studies using animal models and, 2) analyse that data to provide biological information that is not currently available for sheep receiving ECLS following smoke-induced acute lung injury.
Methods

The study was carried out at the purpose-built Medical Engineering Facility of Queensland University of Technology (QUT-MERF) at the Prince Charles Hospital Campus of The University of Queensland [1]. In the original study, sheep inhaled standardised cotton smoke generated by a device that combusts material in an oxygen-deficient environment as previously described [8]. Briefly, 8 g of cotton towelling was combusted in a chamber with transparent walls and 400 ml tidal volume. One tidal volume breath (approximately 10–12 ml/kg) of the smoke was delivered to the sheep via plastic tubing connected to a 1-m-long tracheostomy tube. A fixed number (12) of breaths were given with each load of cotton over a period of approximately one minute. Serial arterial blood gas samples were taken to assess the effect of smoke inhalation, starting at a predetermined time point after the smoke breath cycles.

Physiological data management system

Raw data were obtained from a previous translational study of critical care monitoring of sheep undergoing treatment for smoke-induced acute lung injury involving several separate projects. Data were collected prior to 23 August 2013 and were obtained from two of the scientists who developed the base model [4] as part of a Research Higher Degree project of the author [9, 10]. All data files were in Microsoft® Excel 97–2003 (Microsoft Corporation, Redmond, WA, USA) format and were grouped per sheep and date of the experiment. Data consisted of separate files of real-time physiological data recorded on the hard drives of the monitoring devices (electronically acquired data), and parameters manually recorded by those monitoring the sheep under anaesthesia (manually acquired data), which included data from the electronic monitoring equipment, as back-up if the electronic monitor malfunctioned.

The source study involved 64 sheep, comprising eight experimental groups of eight sheep based on the study’s multiple objectives, subsequent modifications, and later addition of experimental controls. The experimental groups were classified based on: the duration of treatment (2 and 24 hours; E2H and E24H); treatment after smoke inhalation (injury) for 2 and 24 hours (SE2H and SE24H); and treatment after smoke inhalation and transfusion with fresh or stored (aged) blood (SEF24H or SEA24H), respectively. Two additional groups included one group receiving smoke inhalation injury but no treatment (SC24H), and another group that inhaled room air only as the injury (placebo) and no treatment (C24H). Data from sheep involved in the treatment and transfusion studies were not included in the analysis in this study because these data were beyond the scope of this study. Nineteen sheep were included in the present study; data were analysed for 16 sheep with robust data (E24H and SE24H), and included, but not analysed, for three
from groups SC24H and C24H (early observational data). A systematic approach was developed for processing the data.

Manually acquired physiological data workflow

A clone of the master manual data entry spreadsheet was created by removing the formatting and formulas. Several members of the sheep ECMO research team inspected data repeatedly for errors to ensure that all columns, rows, time points, and data points had been copied correctly, including number formats (Figure 1). Redundant columns were removed and data were aligned to experimental time points (Figure 2). While maintaining the same experimental time point header, data from the table in Figure 2 were split and grouped into the following categories: ventilator settings, blood pressure and haemodynamics, fluids and urine output, arterial blood gas values, activated clotting time, anaesthetics, anticoagulants, and ECLS circuit observations.

Electronically acquired physiological data workflow

Raw electronically acquired physiologic monitoring data were inspected for completeness. The data comprised 36 time points: ECLS pump time (min); time of day (h); electrocardiograph (heart rate); arterial blood pressure (mean, systolic, diastolic, heart rate); central venous pressure (mean); pulmonary artery pressure (mean, systolic, diastolic); oxygenator pressure (pre- and post-); capnography (end-tidal carbon dioxide (etCO₂), respiratory rate); pulse oximetry (SpO₂, heart rate); ECLS pump (flow rate, speed); ventilator (mode, frequency, oxygen, pressure control, inspiratory volume, expiratory volume, expiratory minute volume, pressure maximum, mean pressure, positive end-expiratory pressure, plateau pressure, inspiratory resistance, expiratory pressure, pulmonary compliance, inspiratory flow); mixed venous oxygen saturation (SvO₂); and continuous cardiac output (CCO) (Figure 3). The yellow line in Figure 3 indicates the baseline time point and the grey line represents the smoke inhalation time point. It is important to note that there may or may not have been any data at any given point in time.

The electronically acquired physiological monitoring data were inspected for errors and cleaned to provide data for downstream analysis (Figure 4).

Pre-data analysis checks

Data were then subjected to further integrity checks. An important step was to make a plot of data versus time together with descriptive statistics for all data points in the grouped data. At the time of data processing,
the “Descriptive Statistics” tool of Microsoft® Excel 2010 (Microsoft Corporation) did not complete analysis with missing values. Therefore, the data to be analysed were selected and then the “GoTo” tool (F5) was used, and “Special”, “Blanks”, and thereafter, “OK” were selected to identify blanks. The blanks were deleted by positioning the cursor in the blank cell and using the space bar to clear the cell (the “Delete” or “Backspace” keys did not remove the blanks).

After artefact removal and integrity checks, data for individual sheep were placed into six categories: activated clotting time; anaesthetics + inotropes and anticoagulants; arterial blood gas values; blood pressure + ventilation and haemodynamic data; calculated respiratory + haemodynamic variables; and fluids and urine production. Using specially written macros, data were extracted from each experiment and grouped by parameter corresponding to experimental time points. All sheep treatment data were filed by parameter.

Data from the 19 sheep from groups E24H, SE24H, C24H and SC24H (Figure 5) were processed further. Data integrity checks were again performed and repeated by several sheep ECMO research team members, and data were compiled as shown in Figure 6 (note the obliterated cells after removal of data artefacts). The treatment timeline (a) comprised 22 time points for all experiments where sheep received smoke inhalation acute lung injury (SE24H) (b). A trend plot (c) and descriptive statistics panel (d) were useful in data quality control processes for suitability for downstream data analysis and end-user applications.

**Statistical methods**

To meet the second objective, data from the groups, uninjured/treated and injured/treated groups were analysed. The means, medians and standard deviations of the weights of the sheep, where applicable, were tabulated. The physiological parameters of the groups were charted and compared against each other using one-way analysis of variance (ANOVA), where appropriate. Parameters between groups were compared using a paired two-tailed t-test. All p-values were two-sided and p < 0.05 was considered statistically significant. All statistical calculations were performed using GraphPad PRISM 6 software (GraphPad Software, La Jolla, CA, USA).

**Results**

The biodata of the sheep that were used in the current analysis are presented in Table 1. The weights of the uninjured/treated sheep, unlike the injured/treated group, did not pass the D'Agostino–Pearson omnibus test.
normality test; however, there was no significant difference in the weights of the sheep between groups.

**Ventilation**

All animals were intubated and received mechanical ventilation as previously described [1]. Briefly, the initial ventilator tidal volume was set to approximately 10 mL/kg with a respiratory rate of 15 breaths/min, positive end expiratory pressure (PEEP) of 5 cm H₂O, and an initial F\textsubscript{O₂} (fraction of inspired oxygen) of 1.0. These settings were then titrated based on arterial blood gas results. A low tidal volume, high PEEP strategy was used to minimise ventilator-induced lung injury.

Pulmonary compliance decreased in all of the sheep during the course of the experiments, with the injured/treated (SE24H) animals having the most severe and drastic decrease followed by the uninjured/treated (E24H), injured/untreated (SC24) and placebo/untreated (C24) sheep in that order (Figure 7). There was a significant difference (\(p = 0.0013\)) in pulmonary compliance between uninjured/treated and injured/treated groups. The injured/treated sheep had consistently lower Sp\textsubscript{O₂} compared with the other groups, but there was no significant difference in Sp\textsubscript{O₂} readings between the groups. There was an initial increase in etCO\textsubscript{2} followed by a rapid decrease that lessened 15 minutes after the start of treatment. The etCO\textsubscript{2} of the injured sheep continued to trend downward and plateaued in the uninjured groups. There was a significant difference (\(p = 0.0147\)) in etCO\textsubscript{2} between the uninjured/treated and injured/treated groups.

**Blood gases (arterial samples)**

Blood pH varied between the groups. The placebo/untreated sheep had the highest pH while the injured/treated group had the lowest. There was a significant difference in pH between the uninjured/treated and injured/treated groups (\(p = 0.0343\)).

The pCO\textsubscript{2} in all but the uninjured/treated sheep increased initially before plummeting sharply, forming a shallow trough corresponding to 1 hour after the start of treatment, followed by a slight increase before stabilising in all sheep.

There was a gradual decrease in pO\textsubscript{2} in the treated groups of sheep from baseline before decreasing dramatically at the start of treatment with the injured sheep having the most profound decrease (Figure 8). However, there was no significant difference in pO\textsubscript{2} between the uninjured/treated and injured/treated groups.

Haemoglobin [Hb] concentration decreased slightly from baseline before gradually increasing in the injured sheep and remained relatively constant over time in the uninjured sheep. There was a significant difference in [Hb] between the uninjured/treated and injured/treated (\(p = 0.0131\)) groups.
The fraction of oxyhaemoglobin (FO\textsubscript{2}Hb) decreased sharply with the lowest reading at 5 minutes post-injury before returning to near baseline levels within an hour of starting treatment. The injured/treated sheep had a considerably deeper trough in FO\textsubscript{2}Hb level and there was a significant difference ($p = 0.046$) between troughs. There was no change in FO\textsubscript{2}Hb for the uninjured sheep.

The fraction of carboxyhaemoglobin (FCOHb) increased sharply from baseline, peaking at approximately 5 minutes post-injury and decreasing sharply thereafter to the start of treatment before gradually returning to near baseline levels at approximately 6 hours post-start of treatment in the injured sheep. The injured/treated sheep had a higher peak FCOHb than the injured/untreated sheep, although the difference was not significant. There was no change in FCOHb for the uninjured sheep.

The fraction of methaemoglobin (MetHb) increased gradually from baseline, peaking at approximately 5 minutes post-injury and then gradually decreasing to the start of treatment. This was followed by a gradual return to near baseline levels at approximately 6 hours post-start of treatment in the injured/treated sheep. There was no change in MetHb for the uninjured sheep. There was an initial subtle decrease in calculated haematocrit (Hct) before a steady increase in the injured sheep and relatively flat slopes for the uninjured sheep.

Electrolytes

The blood sodium concentration ([Na\textsuperscript{+}]) was relatively stable and there were no significant differences between groups.

There was an initial decrease in the blood calcium ([Ca\textsuperscript{2+}]) level, with the lowest point at approximately 1 hour past the start of treatment before levelling out thereafter in all groups. There was a significant difference in [Ca\textsuperscript{2+}] between the uninjured/treated and the injured/treated groups ($p = 0.0001$). The placebo/untreated and injured/treated groups maintained the highest and lowest levels of [Ca\textsuperscript{2+}], respectively, throughout the experiments.

Blood chloride ([Cl\textsuperscript{-}]) levels remained stable compared with baseline levels during the initial stages and then increased gradually thereafter.

The blood potassium concentration ([K\textsuperscript{+}]) initially decreased compared with baseline levels, reaching a minimum concentration 1 hour after starting treatment and then gradually increased with a peak at approximately 12 hours post-treatment start in all experimental groups. Although the injured/untreated and injured/treated sheep had higher [K\textsuperscript{+}] than the uninjured sheep, the differences were not significant.

Overall, the anion gap decreased gradually, reaching a relatively gentle slope at approximately 6
hours from the start of treatment and did not change significantly, thereafter. There was a gradual decrease in anion gap from baseline during the course of the experiments and there was no significant difference in anion gap between the uninjured/treated and injured/treated groups.

Metabolites

Although there was an increase in blood glucose level [Glu] for the injured/treated sheep after 6 hours of treatment, the change was not significant.

There was an initial decrease in lactate levels [Lac] 6 hours after the start of treatment, followed by a gradual increase for the injured sheep, especially for the injured/treated group. There was no significant difference in [Lac] between the treated groups.

Acid-base balance

There was an increase in the blood base levels [Base (ecf)] that peaked 1 hour post-treatment, followed by a gradual decrease in the untreated group. [Base (ecf)] in the treated groups remained at baseline levels to 1 hour post-start of treatment, before decreasing markedly in the injured/treated sheep. There was a significant difference ($p = 0.0257$) in [Base (ecf)] between the uninjured/treated and injured/treated groups.

Blood bicarbonate concentrations [$\text{HCO}_3^-$] increased initially in the untreated groups before decreasing gradually; however, levels remained higher compared with the treated sheep.

Haemodynamics

There was a gradual decrease in heart rate (HR) during the course of the experiments, with the placebo/untreated groups maintaining a higher HR compared with the injured/untreated, injured/treated, and uninjured/treated groups early in the experiments. There was no significant difference in HR between the uninjured/treated and injured/treated groups.

The mean arterial blood pressure (MAP) decreased early in the experiments before subsequently increasing gradually, peaking at approximately the treatment start time point, before gradually decreasing again in all but the placebo/untreated sheep. The injured/treated groups had a consistently lower MAP compared with the other groups and there was a significant difference in MAP ($p = 0.0058$) between the uninjured/treated and injured/treated groups.

The mean pulmonary artery pressure (MPAP) increased gradually, with the injured/treated group having a consistently higher MPAP. There was no significant difference in MPAP between the
There was an initial, subtle increase in central venous pressure (CVP) that peaked at approximately 1 hour post-injury followed by a decrease that stabilised at approximately 1 hour post-start of treatment. CVP levels in the injured/treated and placebo/untreated sheep were consistently higher and lower, respectively, during the course of the experiments. Mixed venous oxygen saturation (SvO₂) had a lower baseline before eventually rising to a relatively stable and higher level for the treated sheep, and a slightly lower level for the untreated sheep. The injured/untreated sheep maintained a consistently lower SvO₂ compared with the other groups.

Except for the placebo/untreated group, there was a decrease in continuous cardiac output (CCO) from baseline to approximately 1 hour post-start of treatment. There was a significant difference (p = 0.0009) in CCO between the uninjured/treated and injured/treated groups with CCO in the treated groups increasing sharply before plateauing, especially in the uninjured/treated group. There was also a subsequent gradual decrease in CCO in the injured/treated group.

Stroke volume (SV) began to increase 1 hour from the start of treatment for all groups, except for the injured/untreated group where levels remained relatively constant. SV in the injured/treated group began to decrease after 6 hours of treatment, while SV in the uninjured/treated and placebo/untreated sheep increased steadily before decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SV between groups.

Stroke volume index (SVI) began to increase 1 hour after the start of treatment for all groups, except for the injured/untreated group, for which SVI remained relatively constant. SVI in the injured/treated group began to decrease after 6 hours of treatment while SVI in the uninjured/treated and placebo/untreated groups increased before subsequently decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SVI between groups.

While the cardiac index (CI) of the uninjured/treated and placebo/untreated groups remained relatively close to baseline levels, CI the injured/treated and injured/untreated groups declined gradually over the course of the experiments.

After an initial increase in systemic vascular resistance index (SVRI) to approximately 1 hour after the start of treatment, SVRI began to decrease in all experimental groups before plateauing after 12 hours of treatment followed by a gentle increasing trend until the end of the experiments. SVRI in the injured/treated group was consistently below that of the other groups during treatment while that of the injured/untreated group was correspondingly higher. There was no significant difference in SVRI between the groups.
Pulmonary vascular resistance index (PVRI) remained close to baseline levels for all of the groups until 1 hour after the start of treatment when that of the injured groups progressively increased while that of the uninjured groups remained lower with a subtle decrease to 6 hours post-treatment. PVRI in the placebo/untreated sheep remained near baseline levels and the lowest throughout the course of the experiment.

After a small peak corresponding to the start of treatment, right ventricular stroke work index (RVSWI) in the uninjured sheep gradually increased while that of the injured sheep decreased. There was a significant difference ($p = 0.0196$) in RVSWI gap between the uninjured/treated and injured/treated groups.

RVSWI in the placebo/untreated group remained high while that of the injured/treated group was consistently the lowest.

Left ventricular stroke work index (LVSWI) gradually increased in the uninjured/treated and placebo/untreated groups, before plateauing after 12 and 18 hours of treatment, respectively, while LVSWI in the injured/untreated and injured/treated groups of sheep decreased before plateauing at 12 hours and trending upward after 18 hours of treatment. LVSWI in the placebo/untreated group remained consistently higher than in the other groups while that of the injured/treated group was consistently the lowest.

Following a decrease in the coronary perfusion pressure (CPP) from baseline in the smoke-injured sheep, there was a subsequent increase in this parameter prior to a sustained decrease at 18 hours of treatment, followed by another increase. There was a significant difference in CPP ($p = 0.0018$) between the uninjured/treated and injured/treated groups and CCP in the placebo/untreated sheep remained relatively stable after an initial, subtle increase.

There was an initial subtle decrease in arterial oxygen content ($C_aO_2$) from baseline in all groups before a sustained increase in the injured/untreated group, a steady level in the placebo/untreated sheep, and a sharp trough in the injured/treated and uninjured/treated groups. Following the trough, the $C_aO_2$ of the injured/treated group gradually returned to baseline levels while that of the uninjured/treated group continued on a downward trend. There was a significant difference ($p < 0.0085$) in $C_aO_2$ between the uninjured/treated and injured/treated groups.

There was a slight decrease in the oxygen delivery index (DO$_2$I) in all groups to 1 hour of treatment before a further marked decrease, except for the placebo/untreated sheep. There was a significant difference ($p = 0.0013$) in DO$_2$I between the uninjured/treated and injured/treated groups. The injured/treated group had the lowest DO$_2$I compared with the other groups while the placebo/untreated sheep maintained the highest DO$_2$I profile.
The oxygen extraction index (O$_2$EI) decreased in all groups before plateauing at approximately 6 hours after the start of treatment. There was a significant difference ($p = 0.0247$) in O$_2$EI between the injured/treated and uninjured/treated groups. O$_2$EI in the injured/treated and injured/untreated groups was consistently lower and higher, respectively, compared with those of the other groups.

**Fluid input and urine output**

The volume of intravenous fluids administered to sheep in the different experimental groups varied. The injured/treated sheep had the highest fluid requirements while the placebo/untreated sheep required the least. There was a significant difference ($p < 0.0001$) in fluid requirements between uninjured/treated and injured/treated sheep.

The injured/untreated and injured/treated groups produced the least and most urine on average, respectively. There was no significant difference in urine output between the uninjured/treated and injured/treated groups.

**Anaesthetics**

There was a significant difference ($p < 0.0001$) in the amount of alfaxalone required between the uninjured/treated and injured/treated groups. The uninjured/treated group required more alfaxalone on average and the injured/untreated group required the least amount on average. Ketamine requirements differed between groups, with the injured/untreated group requiring the highest amount on average and the injured/treated group requiring the least. There was no significant difference in the quantities of ketamine required between the uninjured/treated and injured/treated groups but significant differences in midazolam requirements occurred between the uninjured/treated and injured/treated groups ($p = 0.0067$).

**Anticoagulation**

There were no significant differences in heparin infusion doses between the uninjured/treated and injured/treated sheep. Heparin requirements for the placebo/untreated group were the lowest. Activated clotting time increased sharply from baseline during pre-treatment and peaked 1 hour after the start of treatment before decreasing sharply and plateauing. There were no significant differences in activated clotting time between groups.

**ECLS circuit observations**
There were significant differences in the ECLS pump speed, blood flow, and pressure differential between the uninjured/treated and injured/treated groups. Pump speed, blood flow, and pressure differential were significantly different ($p = 0.0022$), ($p = 0.0095$) and ($p = 0.0041$), respectively, between the two groups receiving ECLS. These parameters in the uninjured/treated group were consistently higher than those of the injured/treated group.

**Body temperature**

Body temperature in the untreated groups gradually increased from baseline levels and plateaued at approximately 6 hours after starting treatment, and remained higher than for the treated groups. There was no significant difference in body temperature between the treated groups.

**Inflammatory cells and cytokines**

Data were available in abstract form on inflammatory cell infiltration into the lung tissue with a trend toward increased lung injury in the sheep that inhaled smoke, showing damage to the bronchiolar lining and infiltration of inflammatory cells [11–13].

**Discussion**

The results of this study agree with and confirm earlier preliminary observations that ECLS causes a decrease in pulmonary compliance over time [9]. It was expected that the injured sheep would have relatively lower $\text{SpO}_2$ readings compared with the other groups because of episodes of hypotension with hypoxemia, which can affect pulse oximeter function [14]. The relatively low $\text{etCO}_2$ in the injured sheep suggested that the sheep may have hyperventilated, the causes of which were evaluated with respect to reactive oxygen species or superoxide dismutase activity by a team from the source study [15, 16].

The relatively low blood pH in the injured/treated sheep suggested that the sheep tended to metabolic acidosis as the same group of animals also had low $\text{etCO}_2$. This also means that there was no respiratory component contributing to the observed acidosis. The low $\text{pCO}_2$ in the uninjured/treated sheep could be a result of hyperventilation and the high $\text{pCO}_2$ in the injured/untreated sheep suggested that CO$_2$ clearance was curtailed by injury.

The treatment of the sheep contributed to lung injury by causing deterioration of $\text{pO}_2$. The low $\text{pO}_2$ translated to low partial arterial oxygen pressure/inspired oxygen fraction ($\text{PaO}_2/\text{FiO}_2$) ratio, which was much worse in the injured sheep. This finding showed that ECLS contributed to the deterioration of the $\text{PaO}_2/\text{FiO}_2$
The relatively higher levels of [Hb] in the injured sheep suggested that these animals could have been dehydrated secondary to excessive fluid loss from inflammation and increased vascular permeability [17] despite intravenous fluid replacement. However, blood total protein and albumin levels, better predictors of dehydration in sheep [18], were not measured.

The inverse decrease in FO₂Hb relative to FCOHb following smoke injury was expected and agreed with other studies [17, 19, 20]. It has recently been demonstrated that FCOHb is not correlated to the degree of lung injury [17]. The gradual decrease in MetHb was probably caused by the enzymatic activity of methaemoglobin reductase [21] and the higher Hct observed in the injured sheep could have been due to dehydration because HCT was measured by an automated method.

The [Ca²⁺] was lower than the published normal level of 2.4 mmol/L [22]. Stress associated with yarding of the sheep and phosphorus imbalance in feed are the most likely suggested causes of low [Ca²⁺] [23]. Fasting the sheep for 24 hours prior to the experimental procedures could also have contributed to the relatively low [Ca²⁺].

The increase in [Cl⁻] beyond the normal range of 105 – 110 mmol/L [22] during the experiments suggests that the sheep may have developed respiratory alkalosis. Hyperventilation or metabolic acidosis resulting from sustained salivary loss of sodium bicarbonate that was more severe in the injured/treated group may have played a role hyperchloraemia, because Cl⁻ is known to replace HCO₃⁻ in the latter’s loss [24, 25]. Baseline [K⁺] in all of the sheep was below the published normal range of 4–5 mmol/L [22] and this relative hypokalaemia may have been related to low [K⁺] in the diet [26-28]. The normal anion gap with decreased HCO₃⁻ confirmed the presence of hyperchloraemic acidosis in all but the placebo/untreated sheep. The cause of the hyperchloraemia was likely the prolonged administration of 0.9% NaCl.

Although normal [Glu] in ruminants is usually lower than for other species, its relative progressive increase in the injured sheep may have been related to stress and severe pain associated with injury or the development of enterotoxaemia [29, 30]. The relative increase in [Lac] beyond the reported normal range of 1–2 mmol/L in the injured sheep suggested dehydration, trauma, and sepsis [31]. Sepsis, in particular, is a concern with sub-optimal rumen function leading to loss of its buffer effect and increasing numbers of anaerobic bacteria with prolonged hypomotility, such as occurs during long-duration anaesthesia. Therefore, the increases in both [Glu] and [Lac] are consistent with severe injury.

The elevated [Base (ecf)] above +2 mmol/L for most of the first 12 hours in the placebo/untreated and injured/untreated sheep suggested that the sheep were metabolically alkalotic [29] before returning to
normal levels. The relatively low [Base (ecf)] (less than −2 mmol/L) was consistent with HCO$_3$\(^{-}\) loss and the
tendency to metabolic acidosis [29] in the injured/treated sheep. The marked decrease in [HCO$_3$\(^{-}\)] in the
injured sheep was consistent with metabolic acidosis and was more severe in the injured/treated group,
suggesting that ECLS was a contributing factor.

The resting HR of sheep is 50–80 beats/min [22]. In a study that instrumented conscious sheep, the
baseline heart rate was registered as 106 ± 9 beats/min [32]. In the present report, all of the sheep had a
relatively high HR, suggesting that stress and pain were contributing factors. The gradual decrease in HR
during the course of the experiments was consistent with the effects of anaesthesia [22].

In sheep, a mean arterial pressure below 60 mmHg indicates inadequate tissue perfusion [22].
Although the MAP values in the injured sheep were lower than for the uninjured, MAP values were still within
the published normal value of 70 mmHg [22]; the magnitude of injury was again a predictor of how low the
MAP was. Another predictor for the severity of the injury was the mean pulmonary artery pressure, which
was highest for the injured/treated sheep. The baseline values for MPAP were higher than the 17 ± 1 mmHg
reported in another study using sheep [32]. The baseline CVP in all of the sheep in the present report was >
10 mmHg, which was much higher than the 5.5 ± 1.2 mmHg reported elsewhere [32] in instrumented
conscious sheep and a novel finding in this study. The severity of injury and treatment contributed to the
CVP elevations in this study.

There was a benefit of ECLS treatment for SvO$_2$ as it remained high for both the injured/treated and
uninjured treated groups. The consistently low SvO$_2$ in the injured/untreated group was expected because of
the slightly reduced cardiac output in this group; however, this level of SvO$_2$ was still higher than that
reported in other studies [32]. Smoke injury was associated with a sustained decrease in cardiac output in all
of the sheep that were exposed to smoke. As in CCO changes, the SV, SVI and CI all had similar profiles for
the different groups, with the injured sheep having lower values. The decrease in SVRI in all of the sheep
later in the experiments suggested that there was systemic vasodilation. In contrast, the increase in PVRI in
the injured sheep suggested that vasoconstriction was caused by exposure to smoke injury. The exposure to
smoke injury worsened both RVSWI and LVSWI while there was an increase in both parameters in the
uninjured sheep. Reduced RVSWI is associated with poor functioning of the right ventricle [33, 34] and
LVSWI is a reliable parameter for left ventricular function [35].

The reduction in coronary perfusion pressure in the injured/treated, and to a certain extent the
uninjured/treated sheep, suggested that ECLS contributed to the decrease in CPP, in addition to smoke
injury. CPP is an indicator of myocardial perfusion and has been proposed as a drug target during
resuscitation [36]. The observations in the present study support the suggestion that CPP could be used to predict the severity of injury in sheep.

The apparent increase in CaO$_2$ in the injured sheep could have been due to the relative increase in [Hb] secondary to dehydration. The low DO$_2$I in the injured/treated and uninjured/treated groups suggested that ECLS had a contribution, in addition to smoke, based on the relatively higher DO$_2$I in the injured/untreated sheep. Interestingly, the O$_2$EI had a comparable profile to that of the PaO$_2$/FiO$_2$ ratio, and could also be used to predict the contribution of ECLS to smoke-related injury.

The smoke-injured sheep required considerable amounts of intravenous fluids to compensate for the losses from pulmonary exudation and inflammation [17, 19]. The mean urine production in all groups was marginally lower than the published normal of 1.2 mL/h [22] but still considered to be within the acceptable range for this cohort of sheep. The amount of anaesthetic drugs used was considered adequate for the experiments. Heparin infusion was indicated to prolong the activated clotting time to minimise the risk of thrombosis during intravascular procedures [37].

The reduction in the ECLS pump speed, flow, and the pressure differential could have resulted from systemic hypotension contributing to low amounts of blood to the pump. The ECLS was configured such that the centrifugal pump pulled blood from the inferior vena cava and returned it into the right atrium; therefore, if the circulating volume was low, the flow would decrease for a given pump speed and in this case, both rpm and flow dropped. Centrifugal ECLS pumps are known to be preload dependent and afterload sensitive [38], making rpm and flows directly proportional to each other. The reason for the systemic hypotension remains undetermined. It is possible that an unknown pulmonary component or product produced in the smoke-damaged lungs played a role. The body temperature of the sheep was generally within the physiological range.

Certain observations about this study could affect the interpretation of red blood cell indices and their derivatives. For instance, animals differ from humans in that estimated changes in plasma volume is preferably determined by changes in packed cell volume (PCV) or haemoglobin concentration and total plasma protein (TPP) [39–41]. Also, in animals, there is a wider range of normal PCV than TPP [42]. In domestic animal critical care, the change in both PCV and TPP is most useful as a crude index of change in plasma volume [43]. A centrifuge that spins minute amounts of blood for rapid, cost-effective determination of PCV and TPP permits instant adjustments in the animal’s fluid needs. However, measurements of PCV and TPP were not made in the primary study. As with all data that are collected with different objectives, it was considerably tedious to align certain time points with real-time observations made in the laboratory,
especially for the manually input data. There was also no information about pre-anaesthetic blood tests.

An additional limitation relates to the first objective of creating a data management system for tabulating large data sets from human studies using animal models. Because the method has not been validated, it is considered preliminary and further validation studies are required. Also, the numbers of sheep were low and this was especially so in the injured/untreated and placebo/untreated groups, preventing comparisons between the treated and untreated sheep. A further limitation is that cytokine levels, as predictors of lung injury, were not quantified. Using ELISA assays to quantify cytokine levels proved difficult and the cost was prohibitive in the present study. It is partly for this reason that pioneering studies for the development of proteogenomic assays were proposed [44] as an alternative to ELISA to learn from circulating markers of acute inflammation in injured sheep used as models of intensive care, to understand critical illness.

**Conclusions**

The results of this study demonstrated that this preliminary method of raw data processing was effective and helped show that ECLS contributed to further worsening of pulmonary pathology by reducing lung compliance and PaO$_2$/FiO$_2$ ratio. The O$_2$EI changes mirrored those of the PaO$_2$/FiO$_2$ ratio, and decreasing CPP was a predictor of a greater magnitude of cardiopulmonary injury in sheep. These novel observations further understanding of similar pathology in other patients; for instance, in the resuscitation of smoke-injured animals in house or bush fires. A similar data processing approach could be used in evaluating the effectiveness of a given experimental or clinical intervention to further the understanding of the clinical condition being studied, and to aid in the formulation of treatments aimed at improving the survival of animal patients. In veterinary medicine, albeit now a considerably expensive and remote option, ECLS knowledge could complement the treatment of potentially reversible aspiration pneumonia, a secondary complication associated with both *Ixodes holocyclus* toxicity and laryngeal paralysis, in valuable companion animals.

**List of abbreviations**

ANOVA: Analysis of variance

C24H: Control experiment for 24 hours

CaO2: Arterial oxygen content

CCO: Continuous cardiac output

CI: Cardiac index

CO: Cardiac output
CPP: Coronary perfusion pressure
[\text{Hb}]: Haemoglobin concentration
CVP: Central venous pressure
DO_{2}\text{i}: Oxygen delivery index
E24H: Extracorporeal life support for 24 hours
E2H: Extracorporeal life support for 24 hours
ECLS: Extracorporeal life support
ECMO: Extracorporeal membrane oxygenation
etCO_{2}: End tidal carbon dioxide tension
FCOHb: Fraction of carboxyhaemoglobin
FiO_{2}: Fraction of inspired oxygen
FO_{2}\text{Hb}: Fraction of oxyhaemoglobin
HR: Heart rate
LVSWI: Left ventricular stroke work index
MAP: Mean arterial pressure
MERF: Medical Engineering Facility
MetHb: Methaemoglobin
MPAP: Mean pulmonary artery pressure
O_{2}\text{Ei}: Oxygen extraction index
PaO_{2}: Arterial partial pressure of oxygen
PAP: Pulmonary artery pressure
pCO_{2}: Partial pressure of carbon dioxide
PCV: Packed cell volume
PEEP: Positive end-expiratory pressure
pO_{2}: Partial pressure of blood oxygen
PVRI: Pulmonary vascular resistance index
QUT: Queensland University of Technology
QUT-MERF: Queensland University of Technology Medical Engineering Research Facility
SC24: Smoke control experiment for 24 hours
SD: Standard Deviation
SE24H: Smoke injury and extracorporeal life support for 24 hours
SEA24H: Smoke injury, stored blood transfusion and extracorporeal life support for 24 hours
SEF24H: Smoke injury, fresh blood transfusion and extracorporeal life support for 24 hours
SPO$_2$: Blood oxygen saturation
SV: Stroke volume
SVI: Stroke volume index
SvO$_2$: Mixed venous oxygen saturation
SVR: Systemic vascular resistance
SVRI: Systemic vascular resistance index
TPP: Total plasma protein
UQ: The University of Queensland

Competing interests
A previously undisclosed conflict of interest became apparent from a section of adjunct persons within the research group when important early findings of this paper were first presented at an academic milestone seminar at The University of Queensland in August 2013. Therefore, this report comprises work completed during Research Higher Degree studies from September 2012 to 23 August 2013, only.

Author Contributions
The author (SC) was solely responsible for the study design, writing the manuscript, analysing and interpreting the data, final approval of the manuscript, and is fully accountable for the work.

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Figure Legends

Figure 1. Uncleaned manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep

Figure 2. Cleaned and time-point annotated manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep

Figure 3. Uncleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 4. Cleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 5. Data integrity checks and artefact removal of physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 6. Completed extra-corporeal membrane oxygenation (ECMO) treatment data sheet in sheep listed by parameter for further data analysis

a: treatment timeline, b: all experiments where sheep received smoke inhalation acute lung injury (SE24H), c: parameter trend plot, and d: descriptive statistics panel

Figure 7. Pulmonary compliance of smoke and non-smoke injured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

Dotted lines represent error bar margins. Values are presented as mean ± standard deviation.

Figure 8. Arterial oxygen tension for injured and uninjured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

Arterial oxygen tension (pO₂) values are presented as mean ± standard deviation with no error bars shown.
## Table 1 Subject characteristics in treated and untreated groups (control experiments) of sheep

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<th>Length (m)</th>
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**Key:** BSA=Body surface area; E24H=Uninjured sheep treated with extracorporeal life support (ECLS) for 24 hours (uninjured/treated); SE24H= Smoke-induced acute lung injured sheep treated with ECLS for 24 hours (injured/treated); SC24H= Smoke-induced acute lung injured sheep monitored for 24 hours without ECLS (injured/untreated); C24H=Sheep subjected to room air injury as a control for smoke and monitored for 24 hours without ECLS (placebo/untreated).
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Figure 3
**Figure 5**

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**Baseline, 2 hours pre-ECMO and 24 hours of ECMO in S ALI sheep**

**Legend:**
- **a**: Increase in Heart Rate
- **b**: Decrease in MAP

**Table Notes:**
- Mean Heart Rate: 123.396 ± 486
- Standard Error: 1.687 ± 11612
- Median: 116
- Mode: 110
- Pre-Oxygenator Pressure: 95 ± 88725
- Post-Oxygenator Pressure: 95 ± 87553

**Figure 5**

-心电图（ECG）显示心率（HR）增加。
- 血压（MAP）降低。

**References:**
- http://dx.doi.org/10.1101/058511
- bioRxiv preprint first posted online Jun. 12, 2016;
### Baseline and 24 Hours of ECMO in S.ALI Sheep

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**Figure 6**

![Graph showing baseline and 24 hours of ECMO in S.ALI Sheep](image)
FIGURE 7. Pulmonary compliance (Mean ± SD) of smoke and non-smoke injured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls. Dotted lines represent error bar margins.

- **Uninjured, treated (8 sheep)**
- **Injured, treated (8 sheep)**
- **Injured, untreated (2 sheep)**
- **Placebo, untreated (1 sheep)**

**Axes:**
- Y-axis: Pulmonary Compliance (L/cm H₂O)
- X-axis: Duration of treatment (h)

**Legend:**
- Baseline
- Smoke injury
- 5 min post injury
- 1 h post injury
- Treatment started
- 0.25
- 1.0
- 6.0
- 12
- 18
- 24

**Figure 7**
FIGURE 8. Arterial oxygen tension (pO₂) (Mean ± SD with no error bars shown) for smoke and non-smoke injured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls.