Concentration of Apoptotic Factors in Bronchoalveolar Lavage Fluid, as Potential Brain-Lung Oxygen Relationship, Correspond to the Severity of Brain Injury

Dorota Siwicka-Gieroba¹,*, Sylwia Terpilowska², Chiara Robba³,*, Katarzyna Kotfis⁴, Alicja Wojcik-Zaluska⁵, Wojciech Dabrowski¹.

¹Department of Anaesthesiology, Intensive Care Medical University of Lublin, 20-059 Lublin, Poland
²Collegium Medicum, Department of Surgical Medicine with the Laboratory of Medical Genetics, Jan Kochanowski University, 25-369 Kielce, Poland
³Anaesthesia and Intensive Care, Policlinico San Martino, Deputy of the Neurointensive Care Section of European Society of Intensive Care Medicine, 16122 Genova, Italy
⁴Department of Anesthesiology, Intensive Therapy and Acute Intoxications, Pomeranian Medical University in Szczecin, 70-111 Genova, Poland
⁵Department of Physiotherapy, Medical University of Lublin, 20-059 Szczecin, Poland
*Correspondence: dsiw@wp.pl (Dorota Siwicka-Gieroba); kiarobba@gmail.com (Chiara Robba)

Abstract

Background: The mechanism of acute brain injury initiates a cascade of consequences which can directly cause lung damage, and this can contribute to poor neurological outcomes. The aim of this study was to evaluate concentration of different apoptotic molecules in the bronchoalveolar lavage fluid (BALF) in patients after severe brain injury and to correlate them with selected clinical variables and mortality. Methods: Patients with brain injury receiving BALF operation were included in the study. BALF samples were collected within the first 6–8 hours after traumatic brain injury (A) and at days 3 (B) and 7 (C) after admission to the intensive care unit (ICU). Changes in the BALF nuclear-encoded protein (Bax), apoptotic regulatory protein (Bcl-2), pro-apoptotic protein (p53) and its upregulated modulator (PUMA), apoptotic protease factor 1 (APAF-1), Bcl-2 associated agonist of cell death (BAD) and caspase-activated DNase (CAD) were analysed. These values were correlated with the selected oxygenation parameters, Rotterdam computed tomography (CT) score, the Glasgow Coma Score and 28-day mortality. Results: We found a significant increase in the concentration of selected apoptotic factors at admission (A), at day 3 (B) and day 7 (C) after severe brain damage contrasted with baseline level A (p < 0.001, separately). That concentration of selected apoptotic factors was significantly correlated with the severity of the injury and mortality. Conclusions: Activation of different apoptotic pathways seems to be an important process occurring in the lungs of patients in the early phases after severe brain trauma. Levels of apoptotic factors in the BALF correlates with the severity of brain injury.

Keywords: brain injuries; ICU; apoptosis; mortality; oxygenation; brain-lung crosstalk

1. Introduction

Clinical and experimental findings demonstrate the essential role of isolated acute brain injury in peripheral organ and systems failure. The mechanism of acute brain injury initiates a cascade of consequences, which leads to systemic disturbances and poor outcomes [1]. Brain-lung crosstalk is a crucial complex between the brain and lungs, involving various pathophysiological mechanisms [2]. Central nervous system ischemia is one of the crucial mechanism of secondary injury in different types of neurocritical damages [3]. Oxygen therapy in brain injured patients should be considered carefully because hyperoxemia is connected with worse outcome [4,5]. Recent data suggest that formation of reactive oxygen species (ROS), alterations metabolic function disturbances, hyperoxia-induced vasoconstriction are the main reason of poorer outcome. In opposite, some studies hyperoxemia exposure even some benefit [6,7].

Previously, Minabres et al. [8] documented that a apoptosis activation was an independent factor corresponded with 6-months mortality and poor outcome in severe brain injury. However, the molecular mechanisms of lung damage mediated by brain trauma are still under debate. Our previous investigations showed the increase of caspase 3, 6, 8, 9 and 12 concentrations in the BALF of patients after severe brain damage [9]. Therefore we decided to investigate the concentrations of selected proteins involved in the induction of apoptosis.

The primary aim of this study was to evaluate concentration of different apoptotic factors in bronchoalveolar lavage fluid (BALF) after isolated severe brain damage. Secondary aims include to assess the connection between the activation of proteins involved in apoptosis with selected clinical variables and mortality.

We hypothesise that the occurrence of isolated brain injury may lead to an activation of apoptotic pathways in lung, thus representing an evolving lung injury mediated by brain-lung crosstalk, and that the apoptotic proteins detected in BALF may be connected with the severity of injury.
Fig. 1. Flowchart of patient inclusion and exclusion. Figure showed exclusion of 73 patients during intensive care from the study: missing data, lung injury, respiratory bacterial contamination and those who died prior to 7 days. The final study sample included 40 patients.

2. Materials and Methods

2.1 Study Settings

This prospective study was conducted in accordance with the Declaration of Helsinki and applicable regulatory requirements approved by the Bioethics Committee of the Medical University in Lublin, Poland (KE-0254/210/2017).

Inclusion criteria included adult patients (age ≥18), who were hospitalized for severe, isolated traumatic brain injury (TBI) and admitted to the intensive care unit (ICU). Patients on mechanical ventilationsupport during the initial 24 h in the ICU and with isolated brain injury, who had a head computed tomography (CT) scan were included in the study (Fig. 1).

Diagnoses of brain damage were defined according to the Acute Physiology and Chronic Health Evaluation III (APACHE III) and the International Classification of Diseases and Related Health Problems, 10th Revision [10]. Brain injury was classified basing on radiological findings at admission CT.

Exclusion criteria were patients aged under 18 years, pregnancy, patients with history of pulmonary and hepatorenal chronic diseases, with neoplastic diseases and drug-intoxication and prior transplant recipients.

2.2 ICU Monitoring Protocol and Treatment

All patients with severe brain injury between January 2018 and December 2020 included in the study were sedated and treated according to the latest Brain Trauma Foundation guidelines [11]. Our monitoring and treatment methods have been formerly documented [12].

All brain injured patients were mechanical ventilated according to strategies described [1,13]. The PEEP levels (between 5–8 cmH2O) were adapt to preserve values of minimal oxygenation (SatO2) between 94–97%, and PaO2 > 70 mmHg.

Intracranial space-occupying lesions (subdural and/or epidural hematomas), were removed via craniotomy or craniectomy, at the neurosurgeon’s discretion.

2.3 Data Collection

BALF from patients was collected within 6–8 hours after isolated brain injury (A) and at day 3 (B) and 7 (C) after initial damage. A flexible bronchoscope was introduced into the airway and lavages from the middle lobes were achieved using sterile saline (20 mL per lavage). The collected BALF was processed in a standard manner according to described protocols [14]. The collected two millilitres of BALF were centrifuged to separate the fluid from the cells (1900 rpm/10 min/room temperature), and the supernatant was stored at –80 °C for further study.

2.4 Determination of pro-and Antiapoptotic Factor Concentrations

Detection of proapoptotic and antiapoptotic factors concentrations was undertaken by an Enzyme-linked Im-
 munosorbent Assay Kit according to the original instructions of the manufacturer, Cloud-Clone Corp. (Houston, TX, USA). The samples were analysed on microplate reader at 450 nm. A standard curve was created by plotting the absorbance of each standard vs. the suitable standard concentration (Table 1).

2.5 Study Variables

Primary clinical variables were blood analyses, selected inflammatory parameters, oxygenation and blood gas analyses, extravascular lung water (EVLWI) and pulmonary vascular permeability index (PVPI). We categorized PaO$_2$ values as normoxaemia (PaO$_2$ 75–100 mmHg), hypoxemia (PaO$_2$ < 75 mmHg) and hyperoxaemia (PaO$_2$ > 100 mmHg).

Secondary study variables were correlated between apoptotic markers concentration and selected scores systems as Glasgow Coma Score (GCS), Rotterdam computed tomography score (CTS) care system. Data on mortality were received on day 28.

2.6 Statistical Analyses

The appropriate statistical tests were selected after normal distribution testing by plotting and the Shapiro–Wilk test. Continuous variables are analysed using ANOVA analysis of variance with repeated measures. Multiple comparisons between the means of measurements were made using Tukey’s HSD (honestly significant difference) test. The connections between the received values of selected medical indicators on three dates of measurements were correlated using Pearson’s and Spearman’s correlation coefficient. Cox regression was used to calculate the hazard ratio and 95% confidence interval. A p-value of < 0.05 cut off was considered as statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (version 26, IBM SPSS, Chicago, IL, USA) software.

3. Results

3.1 Characteristics of the Patients

The study included 113 adult ICU treated patients with isolated severe brain injury. We excluded 73 patients during intensive care from the study, 31 patients did not meet the inclusion criteria (because of severe lung dis-eases, acute heart failure with pulmonary oedema, lung con-

tusions) (Fig. 1, Table 2).

Finally 40 patients, between 18–76 years old, 24 men and 16 women, were included in study (Fig. 2).

Table 1. Detection range and sensitivities of apoptotic factor assays.

<table>
<thead>
<tr>
<th>Apoptotic factors</th>
<th>Detection range</th>
<th>Sensitivities of the assays (less than)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase Activated DNase (CAD)</td>
<td>156–10 ng/mL</td>
<td>0.054 ng/mL</td>
</tr>
<tr>
<td>Tumour protein p53 (TP53)</td>
<td>78–5.000 pg/mL</td>
<td>27 pg/mL</td>
</tr>
<tr>
<td>B-Cell Leukaemia/Lymphoma 2 (Bcl-2)</td>
<td>0.156–10 ng/mL</td>
<td>0.061 ng/mL</td>
</tr>
<tr>
<td>Bcl2 Associated X Protein (BAX)</td>
<td>0.78–50 ng/mL</td>
<td>0.30 ng/mL</td>
</tr>
<tr>
<td>p53 Upregulated Modulator of Apoptosis (PUMA)</td>
<td>0.156–10 ng/mL</td>
<td>0.056 ng/mL</td>
</tr>
<tr>
<td>Apoptotic Peptidase Activating Factor 1 (APAF1)</td>
<td>0.156–10 ng/mL</td>
<td>0.057 ng/mL</td>
</tr>
<tr>
<td>Bcl2 Associated Death Promoter (BAD)</td>
<td>70–5.000 pg/mL</td>
<td>31 pg/mL</td>
</tr>
</tbody>
</table>

Fig. 2. Age distribution of the patients.

The C-reactive protein were higher at admission in group of non-survivors compared survivors (p = 0.051, F = 4.079), as well as at day 3 and day 7 (p = 0.021, F = 5.769 and p = 0.008, F = 7.845 respectively). Lactate levels were also significantly higher in non-surviving patients (p = 0.046, F = 4.257) compared to survivors.

Non-survivors presented a statistically significant lower value of GCS (p < 0.01, F = 44.109) and a statistically significant higher Rotterdam CT score (p < 0.01, F = 51.282). There was no statistically significant difference in APACHE II scores at admission between the surviving and non-surviving patients (p = 0.113). In addition there were no significant findings between all apoptotic factors and performance of surgical procedures (p > 0.05).

3.2 Oxygen Parameters

The analyses showed that in the non-surviving group of patients, the fraction of inspired oxygen (FiO$_2$) at admission was significant higher (p = 0.002, F = 11.232) compared to survivors. Importantly, the mean values of FiO$_2$ at admission, day 3 (B) and day 7 (C) were higher in non-
Table 2. Patient characteristics at admission (A), at day 3 (B) and day 7 (C), presented by mean value (SD).

<table>
<thead>
<tr>
<th></th>
<th>General</th>
<th>Survivors</th>
<th>Non-survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46 (15)</td>
<td>44 (17)</td>
<td>47 (12)</td>
</tr>
<tr>
<td>CT findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marshal II</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Marshal III</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Marshal IV</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Marshal V</td>
<td>19</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Marshal VI</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Clinical exam.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glasgow Coma Score</td>
<td>4.5 (1.33)</td>
<td>5.29 (1.12)</td>
<td>3.3 (0.47)</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>22 (5)</td>
<td>21 (6)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>Rotterdam CT scale</td>
<td>4 (1)</td>
<td>4 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Lactate during admission*</td>
<td>3.2 (1.37)</td>
<td>2.85 (1.35)</td>
<td>3.73 (1.27)</td>
</tr>
<tr>
<td>CRP A</td>
<td>144.15 (59.19)</td>
<td>129.29 (57.88)</td>
<td>166.44 (55.59)</td>
</tr>
<tr>
<td>CRP B</td>
<td>187.80 (46.15)</td>
<td>174.29 (39.31)</td>
<td>208.06 (49.37)</td>
</tr>
<tr>
<td>CRP C</td>
<td>196.08 (69.68)</td>
<td>172.83 (56.12)</td>
<td>230.94 (75.05)</td>
</tr>
<tr>
<td>ICU length of stay*</td>
<td>32 (43)</td>
<td>45 (51)</td>
<td>14 (4)</td>
</tr>
<tr>
<td>Ventilation days*</td>
<td>26 (34)</td>
<td>36 (41)</td>
<td>13 (4)</td>
</tr>
<tr>
<td>PaO2/FiO2 ratio at admission*</td>
<td>345.9 (116.3)</td>
<td>304.7 (76.3)</td>
<td>407.6 (139.6)</td>
</tr>
</tbody>
</table>

The Marshall score is presented by number of patients. ICU, intensive care unit; FiO2, fraction of inspired oxygen; PaO2, partial pressure of oxygen. * statistically significant difference between survivors and non-survivors.

survivors ($p < 0.001$, $F = 15.294$; $p = 0.001$, $F = 12.106$ and $p < 0.001$, $F = 16.132$ respectively).

In addition, mean PaO2 at admission was significantly lower in surviving patients ($p < 0.001$, $F = 19.196$). There was no significant difference between groups compared to mean PaO2 at day 3 and day 7 ($p > 0.05$) (Fig. 3).

Patients with a higher mean value of PaO2 at admission had significantly ($p < 0.001$) shorter survival times than those with lower PaO2 mean values (hazard ratio 4.410, 95% confidence interval (CI) 1.969; 9.875) (Fig. 4).

3.3 Concentration of Selected Apoptotic Factors Expression Levels, in BALF of Brain Injured Patients at Different Timepoints

The statistically significant increase of selected apoptotic factors such as p53 ($0.037$, BAX ($p = 0.021$), PUMA ($p < 0.001$), APAF-1 ($p < 0.001$), BAD ($p = 0.05$), CAD ($p = 0.002$) between 3 timepoints A, B, C were observed. There was no statistical increase of Bcl-2 between timepoints A and C ($p = 0.171$).

Importantly, in patients who died, the elevation of selected apoptotic proteins such as BAX (Mann-Whitney $U = 1$, $\eta^2 = 0.45$, $p = 0.003$), p53 ($U = 0$, $\eta^2 = 0.48$, $p = 0.002$), PUMA ($U = 6$, $\eta^2 = 0.32$, $p = 0.014$), APAF-1 ($U = 0$, $\eta^2 = 0.48$ $p = 0.002$), BAD ($U = 2$, $\eta^2 = 0.42$, $p = 0.005$) and CAD ($U = 0$, $\eta^2 = 0.49$, $p = 0.002$) were higher at third (B) and seventh (C) day.
Pearson’s correlation with regression analysis documented that concentrations of BAD \( (p < 0.001, r = -0.662) \), CAD \( (p < 0.001; r = -0.579 \) and \( r = -0.602) \) at day 3 and at day 7 and PUMA \( (p < 0.01, r = -0.715) \), p53 \( (p < 0.01; r = -0.619) \), APAF-1 \( (p < 0.01, r = -0.623) \), BAX \( (p < 0.01; r = -0.585) \) and Bcl-2 \( (p < 0.01; r = -0.523) \) at day 7 were significantly associated with the Glasgow Coma Score at admission.

Statistically significant lower GCS scores (3–4 points) were observed in patients with higher activation of selected apoptotic factors at day 7 (BAX \( p < 0.01 \); Bcl-2 \( p = 0.01 \); p53, PUMA, APAF-1, BAD and CAD \( p < 0.01 \) respectively), especially in the non-survivor group.

In addition, internally in the survivor group there was no association between GCS at admission and an increase of selected apoptotic factors at day 3 and day 7 \( (p > 0.05) \).

Importantly, in patients with worse injury (scores of 5–6) assessed on the Rotterdam CT scale at admission, the elevation of factors, in particular BAX \( (p < 0.001) \), Bcl-2 \( (p < 0.05) \), p53, PUMA, APAF-1, BAD, CAD \( p < 0.01 \) respectively) was significantly elevated at timepoints B and C (Fig. 5).

3.4 Correlation between Activation of Apoptotic Factors in BALF of Brain Injured Patients and Selected Clinical Variables

The results of Spearman’s rho correlation showed a significant statistical association between CRP in patients with more severe injury and activation of apoptotic factors in BALF such as PUMA (at admission, \( p = 0.007 \)), BAX, p53, PUMA, APAF-1 and CAD at day 3 \( (p = 0.046; p < 0.05; p < 0.05; p = 0.015; p = 0.016, \) respectively) and APAF-1, BAX, p53 at day 7 \( (p = 0.034; p = 0.046; p = 0.01 \) respectively). Finally, the level of extravascular lung water index (ELWI) was significantly and positively associated with the growth of selected apoptotic factors, except for the Bcl-2 protein (Table 3).

The value of PVPI at admission was positively correlated with an increase in BAX protein \( (p = 0.014; \) moderate relationship) and an increase in p53, PUMA, APAF1, BAD and CAD proteins \( (p < 0.001 \) respectively; strong relationships) (Table 3).

There were no significant statistical findings between apoptotic proteins levels at different timepoints and clinical variables such as, neutrophil-to-lymphocyte ratio, gender, d-dimers, or Apache II score at admission.

3.5 Correlation between Elevation of Apoptotic Factors in BALF of Brain Injured Patients and 28-Day Mortality

Considering 28-day mortality, higher activation of apoptotic proteins such as p53, PUMA, BAX, Bcl-2, APAF-1, BAD, CAD in BALF were documented in patients who died \( (p < 0.001) \). There was no significant association between 28-day mortality and Bcl-2 elevation (Fig. 6).

4. Discussion

4.1 Key Findings

The novelty of this study is the evaluation of the spectrum of apoptotic proteins in BALF after brain injury.

This study shows significant changes in selected apoptotic proteins levels in brain injury patients and documents that different pathways of apoptosis seem to be activated in the respiratory system after brain injury. The apoptotic proteins found in BALF in the 6–8 hours after injury and the increase of these markers over the first 7 days indicate an activated processes of destruction and apoptosis in the respiratory tract over external, internal and oxidative stress pathways.

As far as we know, this is the first human study documenting the status of pro- and antiapoptotic proteins in BALF after brain damage according to severity of injury. In addition, this study presented a significant correlation between oxygen status, apoptotic proteins concentrations in BALF and 28-day mortality.

4.2 Relationship with Previous Studies

The relationship between lung and brain damage remains obscure. Inflammatory mediators, nosocomial infections and adverse effects of neuroprotective therapies increase the vulnerability of the lung to mechanical damage or ischemia-reperfusion insults, increasing the risk of subsequent lung damage [15].

In our study, selected apoptotic factors levels were lower in patients who died compared to survivors during admission to ICU. This relationship was reversed at day 3 and 7. The rapid increase of the expression of selected apoptotic proteins suggests an increased apoptosis rate after brain injury and precised a relationship between selected apoptotic protein levels in BALF and the hardness of injury.

Of course, an important aspect of this apoptotic activation is mechanical ventilation and oxygen administration. Brain damage impacts the mechanics of the respiratory sys-
Fig. 5. Activation of selected apoptotic factors in BALF and severity of damage according to Rotterdam CT score. *p < 0.001 BALF, bronchoalveolar fluid; CT, computed tomography; FiO₂, fraction of inspired oxygen; ** significant change, ° Outliers value.

Fig. 6. Activation of selected apoptotic proteins in BALF according to outcome. ***p < 0.001; **p < 0.01. ° Outliers value.
Table 3. Correlation between activation of apoptotic factors in selected timepoints and EVLW and PVPI in brain injured patients.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Extravascular lung water</th>
<th>Pulmonary vascular permeability index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>BAX</td>
<td>Spearman’s rho</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.012</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Spearman’s rho</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.557</td>
</tr>
<tr>
<td>p53</td>
<td>Spearman’s rho</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.039</td>
</tr>
<tr>
<td>PUMA</td>
<td>Spearman’s rho</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APAF-1</td>
<td>Spearman’s rho</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.003</td>
</tr>
<tr>
<td>BAD</td>
<td>Spearman’s rho</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.006</td>
</tr>
<tr>
<td>CAD</td>
<td>Spearman’s rho</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

It follows that the higher level of ELWI at admission (A), day 3 (B) and 7 (C) corresponded with the greater activation of apoptotic proteins. Statistical significance is marked in bold type. On day 3 (B), PVPI was positively related to BAX, CAD (p = 0.023 respectively; moderate relationship) and PUMA (p < 0.001; strong relationship) proteins increased. Statistically significant correlations were also found between PVPI measured on day 7 (C) and the increase in PUMA, BAD and CAD proteins (p = 0.002; p = 0.049; p = 0.024 accordingly) Moreover, a close to statistical significance was observed (p = 0.059) between PVPI at day 7th (C) and the increase in BAX protein. In summary, the results show that the high PVPI values correlate with a greater increase in selected proteins in BALF.

AAPA-1, Apoptotic Peptidase Activating Factor 1; BAD, Bcl2 Associated Death Promoter; BAX, Bcl2 Associated X Protein; Bcl-2, B-Cell Leukaemia/Lymphoma; CAD, Caspase Activated DNase; ELWI, extravascular lung water; PVPI, pulmonary vascular permeability index; PUMA, p53 Upregulated Modulator of Apoptosis; p53, Tumour protein p53.

It should be mentioned that the higher level of ELWI at admission (A), day 3 (B) and 7 (C) corresponded with the greater activation of apoptotic proteins. Statistical significance is marked in bold type. On day 3 (B), PVPI was positively related to BAX, CAD (p = 0.023 respectively; moderate relationship) and PUMA (p < 0.001; strong relationship) proteins increased. Statistically significant correlations were also found between PVPI measured on day 7 (C) and the increase in PUMA, BAD and CAD proteins (p = 0.002; p = 0.049; p = 0.024 accordingly) Moreover, a close to statistical significance was observed (p = 0.059) between PVPI at day 7th (C) and the increase in BAX protein. In summary, the results show that the high PVPI values correlate with a greater increase in selected proteins in BALF.

APAF-1, Apoptotic Peptidase Activating Factor 1; BAD, Bcl2 Associated Death Promoter; BAX, Bcl2 Associated X Protein; Bcl-2, B-Cell Leukaemia/Lymphoma; CAD, Caspase Activated DNase; ELWI, extravascular lung water; PVPI, pulmonary vascular permeability index; PUMA, p53 Upregulated Modulator of Apoptosis; p53, Tumour protein p53.

It follows that the higher level of ELWI at admission (A), day 3 (B) and 7 (C) corresponded with the greater activation of apoptotic proteins. Statistical significance is marked in bold type. On day 3 (B), PVPI was positively related to BAX, CAD (p = 0.023 respectively; moderate relationship) and PUMA (p < 0.001; strong relationship) proteins increased. Statistically significant correlations were also found between PVPI measured on day 7 (C) and the increase in PUMA, BAD and CAD proteins (p = 0.002; p = 0.049; p = 0.024 accordingly) Moreover, a close to statistical significance was observed (p = 0.059) between PVPI at day 7th (C) and the increase in BAX protein. In summary, the results show that the high PVPI values correlate with a greater increase in selected proteins in BALF.

APAF-1, Apoptotic Peptidase Activating Factor 1; BAD, Bcl2 Associated Death Promoter; BAX, Bcl2 Associated X Protein; Bcl-2, B-Cell Leukaemia/Lymphoma; CAD, Caspase Activated DNase; ELWI, extravascular lung water; PVPI, pulmonary vascular permeability index; PUMA, p53 Upregulated Modulator of Apoptosis; p53, Tumour protein p53.

It should be mentioned that the higher level of ELWI at admission (A), day 3 (B) and 7 (C) corresponded with the greater activation of apoptotic proteins. Statistical significance is marked in bold type. On day 3 (B), PVPI was positively related to BAX, CAD (p = 0.023 respectively; moderate relationship) and PUMA (p < 0.001; strong relationship) proteins increased. Statistically significant correlations were also found between PVPI measured on day 7 (C) and the increase in PUMA, BAD and CAD proteins (p = 0.002; p = 0.049; p = 0.024 accordingly) Moreover, a close to statistical significance was observed (p = 0.059) between PVPI at day 7th (C) and the increase in BAX protein. In summary, the results show that the high PVPI values correlate with a greater increase in selected proteins in BALF.

APAF-1, Apoptotic Peptidase Activating Factor 1; BAD, Bcl2 Associated Death Promoter; BAX, Bcl2 Associated X Protein; Bcl-2, B-Cell Leukaemia/Lymphoma; CAD, Caspase Activated DNase; ELWI, extravascular lung water; PVPI, pulmonary vascular permeability index; PUMA, p53 Upregulated Modulator of Apoptosis; p53, Tumour protein p53.

In addition, animal data has shown that lung slices cultured at 80% oxygen showed significantly stronger cell death activation, antioxidant transcription and acute inflammation [20]. In Our study both FiO₂ and PaO₂ at timepoints A, B, and C were statistically significantly lower in the survivor than in the non-survivor group of patients. This observation may be potentially the reason for higher activation of selected apoptotic factors. Recently, O₂ toxicity has been a matter of concern, especially in regard to lungs and production of reactive oxygen species (ROS). Of late, the continuing discussion about the optimal dosing of oxygen in critically ill patients remains controversial. Of course, elevated FiO₂ in acute brain injury improves brain tissue PbtO₂ but importantly, incremental FiO₂ elevated cerebral excitotoxicity in severe brain damage [21–23].

It should be mentioned that both hyperoxia and hypoxia are associated with worse outcomes in patients with various types of brain injury [24–26]. In recent retrospective studies, hyperoxaemia was significantly associated with increased mortality and poor neurological outcomes [27]. In opposite, in mechanically ventilated patients with subarachnoid haemorrhage Lang et al. [28] did not find correlation between early moderate hyperoxaemia and outcome. Alali et al. [29] documented that PaO₂ between 150–250 mmHg in the first 24 hours after TBI is associated with improvement of long- term functional outcomes. However, this study did exclude patients who died.

Our results join an extensive discussion with the recently published results whether normoxaemia or hyperox-
aemia influence brain function and improve neurological recovery. Optimal PaO₂ targets in daily clinical practise in brain injured patients are currently unknown.

Therefore, oxygen therapy in brain injured patients should be considered carefully, as both hyperoxia and hypoxia are connected with worse outcomes [4,5]. Recent data suggest that the formation of reactive oxygen species (ROS), alterations of metabolic function disturbances, and hyperoxia-induced vasoconstriction are the main reasons for poorer outcomes. In contrast, some studies have documented that hyperoxia exposure presents even some benefits [6,7]. Furthermore, concurrent polytrauma in TBI is also associated with greater systemic inflammation that can breach the blood-brain barrier (BBB) [30].

Decreased alveolar epithelial reliability predisposes to accumulation of oedemic fluid, rich in proteins and cellular material, in the interstitium and alveoli [31]. The management strategy of brain injury patients that aims to increase brain perfusion by fluid administration may worsen pulmonary function because of elevated EVLWI [32]. Several studies documented worse outcome in patients with increased EVLWI and pulmonary microvascular permeability showed as high PVPI [33,34]. We found that the elevation of apoptotic molecules is statistically correlated with the increase of EVLWI and PVPI. A recent study has also demonstrated that high lung microvascular permeability in lung injury is correlated with the activation of apoptotic processes in the lungs [35,36]. The endothelium defender p53 is involved in many respiratory pathologies such as acute lung injury, acute respiratory distress syndrome (ARDS), pulmonary arterial hypertension, pneumonia and tuberculosis [37,38]. Brain injury has also been shown to be able to induce apoptosis of neurons by both p53 dependent and independent pathways [39]. In the case of failure of the p53 protective role, alternative apoptotic mechanisms are activated to enhance apoptosis. In addition, p53 participates in vascular homeostasis and probably in the optimal function of the alveolar-capillary membrane as the blood-air barrier. Importantly, the anti-inflammatory mechanism of p53 may pave the way for a possible therapeutic role in pulmonary diseases [40].

In summary, our results suggest that there is a close relationship between the injured brain and the lungs, that affects the apoptotic processes. The increased of apoptotic proteins in patients after injury, especially in the context of the initial clinical state, ultimately reflects the final outcome.

The lack of a clear mechanism of lung damage after brain injury indicates the complexity of the process and the need for further research. On the one hand, molecules released in the brain, BBB permeability, contribute to the activation of apoptosis processes in the lungs, on the other hand, treatment methods as oxygen supplementation may influences the clinical course after brain injury.

Further studies are required to analyse the transcriptional “software” controlling cell death signalling and lung-brain crosstalk. Thus, from a therapeutic perspective, the apoptotic activity modulators may present a new treatment option for brain injury and concomitant respiratory failure in this group of patients.

4.3 Limitations
This study has several limitations. The modest group of patients may be first and important limitation which significantly reduced the power of our statistical analysis. We also did not study a degree of apoptosis in mechanically ventilated patients without TBI. The degree of neutrophil apoptosis in BALF corresponds to high alveolar levels of chemokine IL-8 followed by increased level of the anti-inflammatory T-helper cytokine such as interleukin 10 [41]. Interestingly, the blockade of alveolar interleukin 10 leads to a lower apoptotic turnover of neutrophils [42]. This process can also be inhibited by dexmedetomidine [43] which is commonly used for sedation also in our patients. Also, massive inflammatory response following surgery- or trauma can dysregulate the pulmonary inflammatory balance intensifying cells apoptosis in alveolar fluid [44]. A lack of more comprehensive evaluation of the cellular components of BALF can also limit our analysis. Unfortunately, no proximity access to advanced laboratory procedures for 24 hours, limit immune cells evaluation.

5. Conclusions
This is first research providing the overview of selected apoptotic proteins concentrations and different apoptotic pathway disturbances in BALF in patients with brain injury. Our data suggest a nearby association between central nervous system injury, oxygen supplementation and apoptosis processes in the lungs, which corresponds to the severity of brain injury.

Availability of Data and Materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions
Study concept and design—DSG, WD, CR. Acquisition, analysis, or interpretation of data—DSG, ST, CR, KK, WD. Statistical analysis—DSG, CR. Tables and figures—DSG, WD. First draft of the article—DSG, ST. Critical revision for important intellectual content—DSG, WD, KK, AWZ, CR.

Ethics Approval and Consent to Participate
All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Medical University in Lublin, Poland (KE-0254/210/2017).
Acknowledgment
Not applicable.

Funding
This research received no external funding.

Conflict of Interest
The authors declare no conflict of interest. Chiara Robba is serving as one of the Guest editors of this journal. We declare that Chiara Robba had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Gernot Riedel.

References


