Pediatric acute respiratory distress syndrome: Host factors in Down syndrome and the general population
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General Introduction

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Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS), is an acute life-threatening pulmonary condition and one of the major challenges of modern intensive care medicine. In ARDS, the permeability of alveolar epithelial and endothelial barriers is increased, due to inflammation and apoptosis. This results in severe hypoxemia and respiratory failure. ARDS was first described by Ashbaugh et al. in 1967 in 12 patients who suffered from acute onset of tachypnea, hypoxemia and loss of lung compliance. Initially called the adult respiratory distress syndrome, it was renamed acute respiratory distress syndrome, when it became apparent that it can occur in children, including neonates, as well. Since its first description, multiple definitions of ARDS were proposed, including the 1994 American-European Consensus Conference (AECC) definition, encompassing hypoxemia criteria (arterial partial pressure of oxygen to fraction of inspired oxygen \( \text{PaO}_2/\text{FiO}_2 \leq 200 \text{ mmHg} \)), the presence of bilateral infiltrates on chest radiograph and no evidence of left atrial hypertension, to exclude cardiogenic pulmonary edema. The entity 'acute lung injury' (ALI) was introduced in the 1994 AECC definition to include patients fulfilling the ARDS criteria, but with less hypoxemia: \( \text{PaO}_2/\text{FiO}_2 \leq 300 \text{ mmHg} \). The 1994 AECC definition has been used for over 18 years, but has its limitations. These include: a lack of explicit definition of 'acute,' sensitivity of \( \text{PaO}_2/\text{FiO}_2 \) to different ventilator settings, poor reliability of the chest radiograph criterion, and difficulties in distinguishing cardiogenic pulmonary edema. In 2012, a new definition for ARDS was proposed (Table 1.1). The term ALI has been omitted from the 2012 ARDS definition, because it led to confusion. Often, ALI was mistakenly used to describe patients with a gas criterion \( \text{PaO}_2/\text{FiO}_2 \) 201-300 mmHg only. The studies presented in this thesis, use the 1994 AECC criteria for ALI and ARDS.

There is no specific therapy available for ARDS in adults nor for ARDS in children, despite numerous experimental and clinical trials and treatment is limited to mechanical ventilation in order to guarantee oxygenation. However, mechanical ventilation in itself may be injurious to the lung, a phenomenon known as ventilator induced lung injury (VILI). Application of lung-protective ventilation (high PEEP and low tidal volume) has been shown to substantially reduce mortality in large randomized controlled trials (RCTs) in adults. In addition, restricted fluid administration in mechanically ventilated patients with ARDS has been shown to reduce mortality. Other RCTs using drugs to reduce inflammation (corticosteroids and anti-cytokine therapies), to reduce pulmonary vascular resistance (prostacycline and inhaled NO), to maintain alveolar aeration (surfactant and β2-agonist) and to reduce diffuse intravascular coagulation (activated protein C) all have failed to show convincing and/or consistent beneficial effects.
TABLE 1.1 The Berlin definition of Acute Respiratory Distress Syndrome (ARDS).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing</td>
<td>Within 1 week of a known clinical insult or new or worsening respiratory symptoms</td>
<td>Chest radiograph or computed tomography (CT) scan</td>
</tr>
<tr>
<td>Chest imaging</td>
<td>Bilateral opacities, not fully explained by effusions, lobar/lung collapse, or nodules</td>
<td>Need objective assessment (e.g. echocardiography) to exclude hydrostatic edema if no risk factor present</td>
</tr>
<tr>
<td>Origin of edema</td>
<td>Respiratory failure not fully explained by cardiac failure or fluid overload</td>
<td>If altitude is higher than 1000 m, the correction factor should be calculated as follows: [Pao2/ FiO2 x (barometric pressure/760)]</td>
</tr>
<tr>
<td>Oxygenation:</td>
<td></td>
<td>If altitude is higher than 1000 m, the correction factor should be calculated as follows: [Pao2/ FiO2 x (barometric pressure/760)]</td>
</tr>
<tr>
<td>Mild</td>
<td>200 mmHg &lt; Pao2/FiO2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm H2O</td>
<td>This may be delivered noninvasively in the mild ARDS group</td>
</tr>
<tr>
<td>Moderate</td>
<td>100 mmHg &lt; Pao2/FiO2 ≤ 200 mmHg with PEEP ≥ 5 cm H2O</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>Pao2/FiO2 ≤ 100 mmHg with PEEP ≥ 5 cm H2O</td>
<td></td>
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</table>

Pathogenesis of ARDS

ARDS is most often caused by a direct hit to the lungs, such as a bacterial or viral pneumonia. However, also non-pulmonary events such as sepsis or severe trauma may be triggers of ARDS. Nevertheless, the central mechanisms and endpoints in the pathogenesis of ARDS are identical, independent of the trigger. These include local dysregulation of inflammation with accumulation of leukocytes and increased permeability of the alveolar endothelial and epithelial barriers (See Figure 1.1).

The neutrophilic granulocyte, also referred to as polymorphonuclear neutrophil (PMN), plays a pivotal role in ARDS pathogenesis. As part of the innate immune system PMNs release proteases, reactive oxygen species and produce chemokines and cytokines. In case of uncontrolled PMN response these defense mechanisms can turn against the host. For instance, neutrophilic migration from blood to the alveolar space may damage the alveolar barrier by lysis of VE-cadherin bonds between endothelial cells, degradation of the extracellular matrix and induction of epithelial apoptosis and necrosis.
The loss of the alveolar barrier results in lung edema, which in itself will hamper gas exchange in the lung, but also reduces surfactant levels, leading to alveolar collapse and decreased lung compliance. This situation is worsened by dysfunction of alveolar type II cells that in normal circumstances produce surfactant and create an osmotic gradient to reabsorb extra vascular water. The beneficial effect of fluid restriction in ARDS is likely based on the effect of lower vascular pressure on transvascular fluid filtration in the presence of...

**FIGURE 1.1** The normal alveolus (left-hand side) and the injured alveolus in the acute phase of acute respiratory distress syndrome (right-hand side). Polymorphonuclear neutrophils (PMNs) migrate through the injured capillary endothelium and alveolar epithelium into the alveolar air space. Alveolar macrophages secrete cytokines and chemokines, inducing further inflammation. Activated PMNs release oxidants, proteases, leading to apoptosis and necrosis of alveolar epithelial cells. The influx of protein-rich edema fluid into the alveolus has led to the inactivation of surfactant. Hyaline membranes form on the denuded basement membrane. Reproduced with permission from Ware LB and Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334-1349, Copyright Massachusetts Medical Society.
increased endothelial permeability. Lung protective ventilation has shown to reduce accumulation of pulmonary edema by persevering the barrier functions of alveolar endothelial and epithelial cells and downregulating mechanosensitive pro-inflammatory pathways.

As mentioned above, excessive PMN activity can lead to increased permeability of the alveolar endothelial and epithelial barriers caused by cell death. There are three major pathways of cell death: necrosis, apoptosis and autophagy. Apoptosis is an important mechanism in the pathogenesis of ARDS, as is shown by histological evidence of increased apoptosis in lung tissue of patients who died from ARDS compared to non-pulmonological disease, and increased markers of apoptosis in broncho-alveolar lavage fluid from patients with ARDS.

Apoptosis is a regulated form of cell death in which the cell-membrane persists long into the process. It is characterized by shrinkage of the cell and its nucleus, in contrast to necrosis, in which the cell and its organelles swell and eventually rupture, resulting in spillage of its contents in the extracellular space. Chromatin condensation, nuclear cleavage and formation of plasma membrane plebs are histological features of apoptosis. The apoptotic cascade is initiated by activation of the death-receptor-pathway, and/or the intrinsic/mitochondrial pathway (See figure 1.2). A third pro-apoptotic pathway is of importance in viral infections, in which cytotoxic T-cells and natural killer cells release perforin and granzymes to target virus infected cells.

Apoptosis of alveolar epithelial cells can be induced by exposure to oxidants. Oxidants have an important role in intra- and extra-cellular signaling pathways and the innate immune system, but can also be harmful to the host when produced in excessive amounts, overwhelming anti-oxidant capacities. In ARDS, oxidants are produced as a result of the inflammatory response by leukocytes, lung epithelial cells and endothelial cells, but can also be a consequence of oxygen therapy. Although anti-oxidant therapies have shown promising results in animal models, clinical trials in patients with ARDS have failed to show convincing results.

**Pediatric versus adult ARDS**

ARDS is much more uncommon in the pediatric population compared with adults (13 versus 79 cases per 100,000 person-years). Mortality from ARDS is relatively low in children, but still estimated to be around 20% in recent epidemiological studies from the USA, Europe and Asia. Each year, between 2500 and 9000 children develop ARDS in the USA alone, of which between 500 and 2000 die. Unfortunately, data on long-term outcome of ARDS in children are not available.
The differences in ARDS incidence, morbidity and mortality between children and adults are intriguing. Especially to the pediatric intensivist, who is partly dependent on knowledge from research in adults to treat his pediatric patients. There are several potential explanations for these differences. First of all, it could be that the clinical definition of ARDS is not specific enough to describe a single disease entity. This is illustrated in a study by Hemptinne and co-workers, who showed that in post-mortem lung specimens of deceased adults who were

**FIGURE 1.2** Pathways of Cellular Apoptosis.

There are two major pathways of apoptosis: the death-receptor pathway, which is mediated by activation of death receptors, and the BCL2-regulated mitochondrial pathway, which is mediated by noxious stimuli that ultimately lead to mitochondrial injury, such as oxygen radicals. Both pathways ultimately activate caspase 3, the key "executioner" caspase. There are numerous pro- and anti-apoptotic regulating proteins. Also, there is potential cross-talk between the two pathways. Reproduced with permission from Hotchkiss RS et al. Cell death. N Engl J Med 2009;361:1570-1583, Copyright Massachusetts Medical Society.
diagnosed with ARDS, only 50% showed diffuse alveolar damage, consistent with ARDS. To further illustrate the potential limitations of the current ARDS definition when used in pediatric intensive care, it is interesting to know that no pediatric intensivists were involved in both the 1994 AECC and the 2012 Berlin definitions of ARDS. Next to a suboptimal definition, three other important variables might help explain the differences in the course of ARDS between adults and children: the event triggering ARDS, the side-effects of treatment and characteristics of the patient, also referred to as host factors.

In both adults and children, pulmonary infections are the most common risk factor for ARDS. However, the pathogens causing the pulmonary infection are not readily comparable between adults and children. For example, in children severe respiratory syncytial virus (RSV) infection is a relatively important risk factor for ALI/ARDS in previously healthy infants. In a study by Dahlem et al. RSV disease was associated with 15% of the children with ALI (Dutch study population: >2 kg body weight and <18 year). Erickson et al. reported that viral lower respiratory tract infection was the cause of ALI in approximately 20% of children (Australian study population: >36 weeks corrected gestational age and <15 years). At the same time, it is important to note that RSV-induced ARDS appears to follow a relatively benign course in infants upon supportive treatment with mechanical ventilation and oxygen therapy, as compared to ARDS induced by other clinical disorders, such as sepsis.

Host factors in pediatric ARDS

The epidemiological differences between children and adults in susceptibility and clinical course of ARDS underscore that age is an important host factors in ARDS. Besides age, other host factors modulating the risk for developing ARDS and its course may differ between children and adults, resulting in differences in morbidity and mortality. For example, in adults, angiotensin converting enzyme (ACE) polymorphism is known to modulate the severity of ALI. However, in a study in 216 children admitted to a tertiary pediatric intensive care unit, the ACE-genotype did not seem to influence the incidence and course of ALI. Furthermore, diabetes mellitus is thought to be protective against ALI in adults, but this has not been investigated in children.

In adults, variants in more than 25 genes have been associated with developing ALI and/or ARDS and with clinical outcomes including common variants of genes that regulate inflammation, coagulation, endothelial cell function, reactive oxygen radical generation, and apoptosis. For example, the Fas pathway modulates apoptosis, inflammation, and epithelial cell injury;
in a candidate gene study, common genetic variants in Fas were associated with susceptibility to developing clinical lung injury.\textsuperscript{45} To our knowledge, these results have not been reproduced in children.

Few studies have focused on differences in pathophysiological mechanisms underlying ARDS between adults and children, but some studies have suggest age-related differential lung responses to injurious events.\textsuperscript{46-47}

\section*{Treatment of pediatric ARDS}

In contrast to adults, there are only a few randomized trials evaluating therapeutic options in children with ARDS. The lack of evidence for treatment of ARDS in children in combination with a potential fatal outcome has led to a situation in which treatment is not protocolized. As a result, the use of different ventilation modalities and drug treatments is highly variable, as shown in a cross-sectional study in 59 PICUs in Europe and North-America.\textsuperscript{48} In most PICUs, lung-protective ventilation is used to prevent or treat ARDS, as this has shown to reduce mortality in adults significantly. Next to protective ventilation, fluid restriction has proven to reduce mortality in adults with ARDS, but again, a randomized controlled trial has not been performed in children. Nevertheless, a recent multicenter observational study concluded that increasing fluid balanced on day 3 in children with ALI is associated with fewer ventilator free days.\textsuperscript{49}

\section*{Down syndrome}

We found that children with Down syndrome (DS) admitted to our PICU fulfill the criteria for ARDS more frequently than controls. DS is the most frequent chromosomal abnormality with an incidence of 1 per 650 live born children in the Netherlands.\textsuperscript{50} DS was first described by John Langdon Down in 1866, but it was not until 1959 that it became known that trisomy of chromosome 21 was the cause of the syndrome.\textsuperscript{51-52} DS is characterized by several physical features, such as epicanthal folds, brachycephaly, downslanted eyes and a flat and broad nasal bridge, combined with mental retardation and hypotonia.\textsuperscript{53} Co-morbidities in DS are common and include congenital heart disease, celiac disease and leukemia.\textsuperscript{54-56}

Children with DS are relatively often admitted to the PICU. A British cohort study showed that children with DS accounted for 1 in 26 admissions.\textsuperscript{57} For short admissions the ICU mortality in DS is comparable with non DS children, but for admissions >10 days, the risk for death is increased in DS.

In DS, both regulation of inflammation and apoptosis are abnormal, which
is thought to attribute to increased risk of infectious diseases and Alzheimer’s disease.\textsuperscript{56-59} Genes that are overexpressed on chromosome 21 are associated with oxidative stress and neuronal apoptosis. However, intervention trials using antioxidant supplements or diets have failed to produce uniform therapeutic effects.\textsuperscript{60} We hypothesized that high levels of oxidative stress in DS results in an increased level of apoptosis and inflammation in respiratory epithelium that may contribute to the increased risk of ARDS in children with DS.

**Outline of the thesis**

The principal aim of this thesis was to investigate the importance of host factors, as opposed to triggering event or treatment effect, in modulating the risk for developing ARDS and modulating disease severity in children.

This thesis was initiated by the clinical observation that children with Down syndrome (DS) are at increased risk for developing ARDS. This observation has been confirmed by an epidemiological study described in Chapter 2. We formulated several hypotheses to explain the increased incidence of ARDS in DS. First, we hypothesized that pulmonary epithelial cells have an increased tendency to undergo apoptosis and investigated this in fetal lung tissue (Chapter 3). Furthermore, in DS, both regulation of inflammation and apoptosis, important in ARDS pathophysiology, are abnormal. This has been linked to an imbalance in free radical scavengers. We measured the expression of free radical scavengers and the effect of oxidative stress in terms of apoptosis and inflammation in respiratory epithelium from children with DS compared to controls (Chapter 4).

In response to findings in adults that increased plasma levels of C-Reactive protein, a marker for inflammation, are associated with favorable outcome,\textsuperscript{61} we explored this association in our pediatric population with ARDS (Chapter 5).

Besides DS, other host factors in pediatric ARDS were studied, in collaboration with other members of the pediatric ARDS research group of the Emma Children’s Hospital/AMC. In Chapter 6, the association between polymorphism of the angiotensin-converting enzyme gene and outcome in respiratory syncytial virus-induced ARDS is described. In contrast to adults, respiratory syncytial virus infection is an important cause for ARDS in the pediatric population.\textsuperscript{39} In Chapter 7, we describe that the tumor-necrosis-factor related apoptosis inducing ligand (Apo2L/TRAIL), which mediates lung injury in adult ARDS, may also be of importance in RSV-induced ARDS in children.

In Chapter 8, the results of the studies presented in this thesis are summarized and discussed, and suggestions for future research are proposed.
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