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Pediatric Acute Lung Injury

Peter Dahlem

PEDIATRIC ACUTE LUNG INJURY

Peter Dahlem

Pediatric acute lung injury.

Thesis, University of Amsterdam, Amsterdam, The Netherlands

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door

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Faculteit der Geneeskunde

*Für meine Eltern
Aan mijn ouders*

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Pediatric Acute Lung Injury

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ABSTRACT

Among ventilated children, the incidence of acute lung injury (ALI) was 9%; of that latter group 80% developed the acute respiratory distress syndrome (ARDS). The population-based prevalence of pediatric ARDS was 5.5 cases /100000 inhabitants. Underlying diseases in children were septic shock (34%), respiratory syncytial virus infections (15.9%), bacterial pneumonia (15%), near-drowning 9%, and others. Mortality ranged from 18% to 27% for ALI (including ALI-non ARDS and ARDS) and from 29% to 50% for ARDS. Mortality was only 3%-11% in children with ALI-non ARDS. As risk factors, oxygenation indices and multi-organ failure have been identified. New insights into the pathophysiology (for example the interplay between intraalveolar coagulation/fibrinolysis and inflammation and the genetic polymorphism for the angiotensin-converting enzyme) offer new therapeutic options. Lung protective mechanical ventilation with optimal lung recruitment is the mainstay of supportive therapy. New therapeutic modalities refer to corticosteroid and surfactant treatment. Well-designed follow-up studies are needed.

INTRODUCTION

Clinical research on acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) is dominated by studies performed in adult patients [1;2]. For example, a Medline search including the terms “Respiratory Distress Syndrome, Adult” OR “acute lung injury” limited to clinical studies resulted in 9607 clinical investigations (date: 25-1-2007); however, when further limited exclusively to children only 762 hits remained. Therefore, our aim was to review the most relevant publications on pediatric ALI.

METHODS

We included relevant publications on children aged from 4 weeks to 18 years suffering from ALI accessible on the National Library of Medicine’s Medline database. ALI/ARDS were defined following the criteria recommended by an American-European Consensus Conference in 1994 (Table 1) [3]. Preference was given to randomized controlled clinical trials (RCT) or nonrandomized case-control studies pub-

lished up to 31 December 2006. Studies involving meta-analyses and systematic reviews were also reviewed. Investigations on pediatric acute hypoxic respiratory failure (AHRF) were only considered if sufficient information was given to apply ALI criteria to the analysed patients. Where appropriate, we have included investigations performed in adult patients with ALI/ARDS and newborns with the respiratory distress syndrome (RDS).

HISTORY OF ALI/ARDS AND DEFINITION

In 1967 Ashbaugh et al. introduced the term “adult respiratory distress syndrome” (ARDS) for a spectrum of conditions characterized by severe hypoxemia, reduced lung compliance and new bilateral infiltrates on chest radiograph (Figure 1), caused by an unrelated underlying critical illness such as, for example, sepsis or aspiration pneumonia [4]. However, the criteria were not clearly defined. Therefore, in 1993 an American-European Consensus Conference (AECC) provided a new definition of ALI and ARDS (Table 1) [3]: “a syndrome of inflammation and increased permeability that is associated with a constellation of clinical, radiological, and physiological abnormalities that cannot be explained by, but may co-exist with, left atrial or pulmonary capillary hypertension” ... and “is associated most often with sepsis, aspiration, primary pneumonia, or multiple trauma and less commonly with cardiopulmonary bypass, multiple transfusions, fat embolism, pancreatitis, and others.

Figure 1. A child with meningococcal septic shock. Day 1: the left-hand image shows bilateral infiltrates. Day 3: the right-hand image shows progression to generalized infiltrates with pleural effusions.

Table 1.

The American-European Consensus Conference definitions of ALI and ARDS

Oxygenation	ALI	$\text{PaO}_2/\text{FiO}_2 < 300$ (regardless of positive end-expiratory pressure level)
	ARDS	$\text{PaO}_2/\text{FiO}_2 < 200$ (regardless of positive end-expiratory pressure level)
Chest radiograph		Bilateral infiltration seen on frontal chest radiograph
Pulmonary artery occlusion pressure		< 18 mm Hg when measured, or no clinical evidence of left atrial hypertension

From that moment onwards ALI came to represent the entire spectrum of this condition, and ARDS was reserved to apply to patients with more severe hypoxemia. Because children may also be affected, the AECC changed the “A” previously referring to “adult”, to the “A” referring to “acute” (respiratory distress syndrome) [4-6].

PATHOPHYSIOLOGY

Independently of age, ALI is characterized by an initial insult, which triggers cell-mediated mechanisms releasing a cascade of a variety of mediators. They disturb the integrity and function of the cellular linings of the alveolar-capillary unit (Figure 2).

Figure 2. An illustration of the pathophysiology of acute lung injury (adapted from reference 1).

Independently of age, ALI is characterized by an initial insult, which triggers cell-mediated mechanisms releasing a cascade of a variety of mediators. They disturb the integrity and function of the cellular linings of the alveolar-capillary unit (Figure 2). Hyaline membranes, flooded alveoli with protein-rich edema fluid, infiltrates of polymorphnuclear neutrophils (PMN), macrophages and erythrocytes are the leading histological hallmarks of ALI (Figures 3a and 3b) [7;8]. Pro-inflammatory mediators are expressed in lung alveolar and endothelial cells, which are associated with the onset, severity and course of ALI [9]. The degree of inflammation depends on the biologic activity and the imbalance between pro- and anti-inflammatory cytokines, for example interleukin (IL)-8 versus IL-1 [9;10].

A polymorphism in the gene encoding angiotensin-converting enzyme (ACE) is linked to the susceptibility and outcome of ARDS [11]. ACE cleaves angiotensin I to generate angiotensin II, which stimulates the production of pro-inflammatory medi-

ators like IL-8 and IL-6 in alveolar epithelial cells [12]. Furthermore, angiotensin II is a potent vasoconstrictor and a key factor in the Fas-induced apoptosis (programmed cell death) of alveolar epithelial cells in vitro [13;14]. Animals with ALI that were deficient for ACE had reduced pulmonary edema formation and leukocyte infiltration [15].

Similar to bacterial sepsis, a close interrelationship exists between inflammatory mediators and the coagulation cascade in ALI [16;17]. Activation of pro-coagulative factors (tissue factor) and inhibition of fibrinolysis (plasminogen activator inhibitor (PAI)-1) have been identified to produce platelet–fibrin thrombi in small pulmonary vessels [18;19]. This interplay occurs both intra- and extravascularly, and in the alveolar compartment [18;20–22]. Unresolved fibrin depositions and alveolar hyaline membranes are the net result [23–25]. Surfactant function is inactivated by plasma proteins leakage and its production is further diminished by damage of pneumocyst type II [26].

For overall resolution (end of the first week) the dynamic interaction between inflammation, coagulation, restoration of water transport and cell function need to be rebalanced and surfactant production restarted [27]. The clearance of pulmonary edema fluid and transcapillary water transport are crucial [1;10]. Apoptosis should be rebalanced providing the clearance of inflammatory cells (e.g. PMN). The exact mechanisms of repair are still under investigation (Figure 4) [28–30].

Unfortunately in some patients resolution is hampered. Histologically, these patients show alveolar fibrosis along with persistence of inflammatory cells and only partial resolution of pulmonary edema [31]. Transforming growth factor beta causes persistent depression of fibrinolysis and formation of fibrin depositions [32–36]. This pro-fibrosing milieu may start already early in the course of ALI [37;38]. On the long term, permanent abnormalities in respiratory function and reduced health-related quality of life are observed [34;39–41].

Figure 3. Illustration showing the normal alveolus and (Figure 3a) and the injured alveolus (Figure 3b) during acute lung injury (ALI).

In the acute phase of ALI (Figure 3b) there is formation of protein-rich hyaline membranes on the denuded basement membrane. Neutrophils are migrating through the interstitium into the air space. Alveolar macrophages secrete interleukin-1, 6, 8, and 10, as well as tumor necrosis factor alpha, which stimulate and activate neutrophils. Neutrophils release pro-inflammatory molecules (oxidants, proteases, leukotrienes, platelet-activating factor). The influx of protein-rich edema fluid into the alveolus inactivates surfactant and, unresolved fibrin depositions form fibrin-rich hyaline membranes.

Figure 4. Illustration of the recovery from acute lung injury.

During recovery *de novo* proliferation and differentiation of alveolar type II cells occurs. For complete resolution it is important that water is moving via additional aquaporins and by recovery of sodium and chloride channel function (ENaC) and sodium pump ($\text{Na}^+/\text{K}^+-\text{ATPase}$). Also intraalveolar protein must be cleared by paracellular diffusion and secondarily by endocytosis. Furthermore, insoluble debris (protein, apoptotic neutrophils) is removed by macrophages. Very important for complete recovery is the gradual remodeling and resolution of intraalveolar and interstitial granulation tissue and fibrosis (left hand side of figure, adapted from reference 10).

Lung mechanics

ALI/ARDS in adult patients are characterized as a restrictive disease with reduced lung compliance caused by loss of surfactant function, atelectatic lung regions and accumulation of interstitial/alveolar plasma leakage [6]. Newth et al. confirmed reduced lung compliance in children with ARDS, and the decrease in lung compliance correlated with severity [42]. Respiratory function measurements at the bedside might help to identify patients with more severe disease. However, appropriate respiratory function devices are not routinely used and are not available on most of the pediatric intensive care units (PICU). Therefore, implementation of this technique in daily routine has not yet occurred.

EPIDEMIOLOGY

Incidence

About 40% of critically ill children admitted to a PICU are mechanically ventilated, and about 14% of them are suffering from AHRF [2;43]. Before the AECC publication in 1994, only a limited number of retrospective studies paid attention to epidemiological data on children with AHRF or ARDS.

Based on the AECC criteria, we have identified only four studies performed on a PICU and only one population-based study performed in Germany [43-47]. In those five studies, among the mechanically ventilated children the incidence of ALI was 9%, and 80% of that group developed ARDS resulting in an incidence of 7% to 8% [43;47]. In relation to all PICU admissions the incidence of ARDS was calculated to be 3% to 4% [43;46]. The only population-based study was conducted in a German district and reported a prevalence of pediatric ARDS of 5.5 cases/100,000 inhabitants and an incidence of 3.2 cases per year/100,000 inhabitants [44]. In contrast, a much higher incidence of ALI was found in adults ranging from 18 to 86 cases per year/100,000 inhabitants [48-50]. In general, comparisons between studies are difficult and depend on the characteristics of the study population enrolled [43]. For example, in the report by Randolph et al. of eight PICUs in North America, three included medical/surgical patients, and five included medical, surgical and cardiac patients [47].

Gender

It was speculated whether higher testosterone levels in male children might predispose them to critical illness [51;52]. Data from the ESPNIC ARDS database (www.meb.uni-bonn.de/ards) showed that more boys than girls developed ARDS (45 vs.27) in the age group $0 < 12$ months [53]. Therefore, it was concluded that factors other than testosterone might be involved in the male preponderance (low levels of male sex hormones at this age). It is likely that differences in lung mechanics between male and female infants (with disadvantages to the male) might contribute to ALI/ARDS [54].

Mortality in children versus adults

Overall mortality in children suffering from ALI (including ALI-non ARDS and ARDS), ranged from 18% to 27% and, not surprisingly, mortality increased to 29%-50% when children developed ARDS. In contrast, mortality was only 3% to 11% in those who did not develop ARDS [2;43;45]. In adults with ALI mortality was higher; the in-hospital mortality rate was 38.5% and mortality increased with age up to 60% [48-50]. Interestingly, for adult patients no differences between those who developed ARDS and those who did not develop ARDS (i.e. ALI-non-ARDS) were found [48].

Mortality in children with ARDS has been associated with multi-organ failure; for example as demonstrated by the Pediatric Risk of Mortality Score (PRISM, a validated score to predict mortality of children immediately after admission on a PICU) [43;45;46]. As cause of death neurological and/or cardio-circulatory failure rather than respiratory failure was identified. In general, independently of the severity of ALI/ARDS, a worse course was determined based on the past medical history and the primary underlying disease (e.g., immunosuppression, severe cerebral injury, inborn error of metabolism) [43;45]. Therefore, it has been proposed that "the presence of severe pre-existing disease or associated pathology, rather than severity of respiratory failure alone is associated with outcome" [2].

Risk factors

Studies reported a correlation between the $\text{PaO}_2/\text{FiO}_2$ ratio on day one and/or its further deterioration and mortality [43;45]. However, other investigators were not able to confirm these findings and there is still controversy about respiratory variables and their association with outcome parameters [2;45;55]. Much interest was also directed to biological markers, and whether or not they may correlate with outcome. Although the results have been inconsistent, their validity improved when biological markers were used in combination with the underlying clinical condition (e.g. sepsis) [55].

The factors which predispose the individual patient at risk are not yet understood and the search for gene candidates has been challenged [56;57]. For example, it was demonstrated that the DD genotype for ACE was associated with increased mortality in ALI patients [58;59].

Despite these inconsistencies, efforts should still be made to identify prognostic factors for use in the early phase of ALI in order to define which patient might benefit most from novel therapeutic strategies. Independently of the possible risk factors associated with ALI, in some situations the underlying disease may determine outcome. For example, in one study 2 of 4 children and in another study 83.3% of children with immunodeficiency died [43;53]. Dahlem et al. found that 7 of 11 patients who died had irreversible cerebral damage, which might have had a considerable impact on outcome [43]. Therefore, it was suggested that it is not helpful in all circumstances to include these categories of patients when calculating the risk factors for ALI [53].

Underlying diseases

In adult patients, a variety of critical diseases may originate ALI (Table 2) [10]. The outcome differs depending on whether the origin of lung injury was caused by direct (e.g. aspiration pneumonia) or by indirect (e.g. sepsis) lung injury [60].

With some differences compared to adult patients, the common underlying diseases in children can be divided into indirect lung injury e.g. caused by septic shock (up to 34%) and direct lung injury caused by pulmonary disorders such as respira-

tory syncytial virus infection (15.9%) and bacterial pneumonia or aspiration pneumonia (up to 15%). Less frequent are a variety of other conditions, including near-drowning (9%), cardiac diseases, oncologic disorders and multi-trauma patients [43;45;61].

Table 2: Disorders causing acute lung injury in adults.

Direct lung injury	Indirect lung injury
Common causes	
Pneumonia	Sepsis
Aspiration of gastric contents	Severe trauma with shock and multiple transfusions
Less common causes	
Pulmonary contusion	Cardiopulmonary bypass
Fat emboli	Drug overdose
Near-drowning	Acute pancreatitis
Inhalational injury	Transfusions of blood products
Reperfusion pulmonary edema after lung transplantation or pulmonary embolectomy	

Complications

Complications directly related to the severity of lung injury are rare and include pneumothorax (8-9%) and failure of conventional ventilation. In these situations of untreatable respiratory failure, alternative and experimental treatment options are applied (Table 3) [43;45;62].

Table 3. Additional treatments in children with AHRF [47].

	Bronchiolitis	Pneumonia	ARDS	All
Numbers	81	48	23	152
Prone position	13.6%	10.4%		
High frequency ventilation	2.5%	8.0%	52.2%	11.8%
Nitric oxide	3.7%	0.0%	17.4%	4.6%
Extracorporeal life support	1.2%	4.2%	8.7%	3.3%
Surfactant	2.5%	0.0%	0.0%	1.3%
Pulmonary artery catheter	0.0%	4.2%	0.0%	1.3%

TREATMENT

The mainstay to treat hypoxic failure is controlled oxygen supply with or without mechanical support. Prevention of fluid overload and measures to stabilize circulation and the patients' comfort (by sufficient pain relief and sedation) are essential supportive targets. In critically ill patients the metabolic balance is shifted towards catabolism and protein malnutrition. Early aggressive nutritional support will help the patient to recover faster [63;64]. The following sections address conventional and novel treatment options for children with ALI.

Mechanical ventilation and Ventilator-Induced Lung Injury

Mechanical Ventilation

When conventional mechanical ventilation was introduced for the treatment of respiratory failure in the 1960s and 1970s, tidal volumes (V_t) of 10-15 mL/kg actual body weight were recommended to maintain arterial CO_2 values within the normal range. However, life-threatening complications such as pneumothorax occurred and mortality was high [65]. With the introduction of positive end-expiratory pressure (PEEP), oxygen uptake improved and the incidence of pneumothoraces in ARDS patients was reduced dramatically [66-68]. In addition, Hickling et al. observed that ventilation with a lower V_t (i.e. instead of 10-15 mL/kg actual body weight) reduced further mortality [69;70].

In parallel with these clinical observations, patho-anatomical and computerized tomographic studies in the 1970s/1980s informed physicians about the uneven distribution of aerated areas and dense consolidated regions of the lung [71]. In adult patients with ARDS, the remaining alveolar surface for gas exchange (i.e., functional residual capacity, total lung volume) was largely reduced. Gattinoni et al. introduced the term “baby lung” to symbolize this condition [72]. “Normal” Vt of 10-15 mL/kg may cause a dramatic overdistention of the “baby lung” resulting in loss of compliance due to the pressure/volume relationship of the lung (Figure 5). Based on these observations the concept of ventilator-induced lung injury (VILI) evolved during the 1990s.

Figure 5. The hysteresis of the pressure-volume curve of the lung. A, inflation limb; B, deflation limb. On inflation (A) lungs need higher pressures to inflate than on deflation. On deflation (B), the higher lung volume can be maintained on lower pressure. Thus, once the lung is open it is more compliant. At the lower inflection point the lung opens up, compliance improves and at the upper inflection point optimal lung volume is achieved. At overinflation, compliance decreases and lung injury occurs.

Ventilator-induced lung injury

VILI is now considered to initiate and to sustain lung injury following a similar histomorphological and inflammatory pattern to that of the original ALI [73-75]. VILI also contributes to multi-organ failure and death. The basic mechanisms of VILI can be

summarized as “barotrauma, volutrauma, atelectrauma and biotrauma” [73].

Barotrauma is characterized by the fact that mechanical forces (high pressure inflation) during artificial inflation cause pressure-related “shear forces” on inhomogeneous (partly aerated and partly consolidated) lung tissues [74]. On microscopy, lungs show disruption of the alveolar capillary sheets with air leaks.

Atelectrauma is defined as repetitive opening and closure of alveolar units during mechanical ventilation with alveolar-capillary stress failure.

Volutrauma: Large V_t cause disruption of alveolar-capillary sheets, pulmonary edema, increased alveolar-capillary permeability, alveolar-capillary stress failure, and structural abnormalities on electron microscopy [76;77].

Biotrauma: Slutsky et al. and others developed the “biotrauma” hypothesis, which addressed the question why do mechanically ventilated patients with ALI die [75;78;79]. VILI shows biochemical characteristics similar to those of original ALI [75;80]. Invasion of PMN and the presence of pro-inflammatory cytokines (e.g., tumor necrosis factor alpha, IL-6, IL-10) play a major role. Additionally, mechanical stretch activates many signal transduction pathways (e.g. mitogen-activated pathway), which activate inflammatory mediators. However, the exact relations between pro-inflammatory and anti-inflammatory mediators and their balance are still under debate: they might differ in children and may occur in healthy as well as in pre-injured lungs (e.g. sepsis-induced ALI) [74;76;81]. Finally, these pro-inflammatory mediators may spill over from the pulmonary compartment to the systemic circulation and trigger a generalized inflammatory response in major organs leading to multi-organ failure and death [73].

Protective ventilation strategies

In order to prevent VILI, lung protective ventilation strategies should aim to prevent atelectasis, re-open atelectatic regions and keep the lung open. Furthermore, overdistention should be prevented and high-pressure ventilation avoided. It has been shown that atelectrauma (alveolar collapse) could be prevented by sufficient PEEP above the lower inflection point in combination with recruitment maneuvers

(Figure 5) [62;82-84]. Furthermore, optimal PEEP has shown to reduce biotrauma by preventing translocation of cytokines (or even bacteria) from the alveolar compartment to the systemic circulation. Without PEEP, however, this compartmentalisation is lost and inflammatory mediators as well as pathogenic microbes are distributed to other organs and cells contributing to secondary sepsis, multi-organ failure and death [73;75;80;85].

Despite all the experimental rationales for applying sufficient PEEP, recently published clinical results have questioned the benefits concerning final outcome. In a RCT of low versus high PEEP, the high PEEP group (13 cmH₂O) disappointingly did not show a significant reduction of mortality [86]. One reason why differences between the groups were not significant might have been the surprisingly low overall mortality of below 26% due to “lung protective” basic ventilator settings in accordance with the ARDS Network recommendation: Vt <6 mL/kg and positive inspiratory pressure (PIP) < 30 cmH₂O. Therefore, additional benefits from a single parameter such as PEEP might have been difficult to demonstrate. Second, a PEEP level of 13 cmH₂O might have been not high enough. It has been demonstrated that PEEP levels above 15 cmH₂O are necessary to keep alveoli open and prevent lung injury [87;88]. Third, when optimal PEEP was combined with small Vt of less than 6 mL/kg and recruitment maneuvers, the outcome of patients improved [89;90].

Despite some limitations in study design, the ARDS Network study set a new ventilation strategy as gold standard [89]: Sufficient PEEP (titrated on FiO₂ or respiratory function measurements), lung recruitment, avoiding PIP above 30 cmH₂O, and Vt not exceeding 6 mL/kg ideal body [82;83;91]. Furthermore, Gattinoni et al. recommended to calculate the Vt not in relation to ideal body weight but in relation to the actual (i.e. smaller) lung volume which can be measured by computed tomography [72]. Using this approach, the levels of inflammatory markers as well as mortality in adult patients have been reduced [78;79;89].

However, reliable data from children with ALI are lacking. Even so, it is questionable, whether evidence from adult patients can also apply to children; we have to acknowledge that there are age-dependent differences in e.g. respiratory tract

anatomy, physiology, underlying diseases, etc. However, conducting a well-designed trial in the pediatric age group will be difficult due to the smaller numbers of children with ALI. Moreover, the evidence and practice derived from adult patients have already entered and are being applied in the PICU.

Despite all the scientific evidence for lung protective ventilation strategies (outside of clinical trials), overall improvement will only be achieved when physicians are aware of these principals in daily routine [92-94].

FURTHER SUPPORTIVE TREATMENT STRATEGIES

High-frequency oscillatory ventilation

High-frequency oscillatory ventilation (HFOV) reduces V_t to a minimum and applies a continuous distending pressure above PEEP. With HFOV, ventilation in the so-called safe zone of the pressure/volume curve can be established preventing volutrauma and atelectrauma (Figure 5). Therefore, one might expect that with HFOV important goals of lung protective ventilation (preventing physical injury due to repetitive closure and re-opening of alveoli) may be achieved. Most of the clinical HFOV studies were conducted in newborns with neonatal RDS; however, no clear benefit on mortality compared with conventional ventilation (CV) was observed [95-99].

In adult patients with ALI, reviews by leading experts in the field and a Cochrane systematic review of RCTs found that HFOV during the early phase of ALI seems to have no benefits and that there might be a role for HFOV only in severe ARDS [99-101].

For pediatric ALI/ARDS, only one prospective RCT on HFOV has been conducted [95]. It was found that physiological parameters, oxygenation and lung recruitment improved. However, the duration of mechanical ventilation and 30-day mortality did not differ between the HFOV and control group. Therefore, no recommendation for routine use can be made. Despite lacking sufficient evidence, the frequency of its use varied from occasionally up to almost 30% [43;102].

Prone position

During prone position, dependent lung regions are recruited under the influence of gravity. Promising clinical observations in adult ALI/ARDS patients demonstrated that prone position improves oxygenation by 70–80% [103-106]. A trend towards lower mortality was observed when patients suffered from severe ARDS and when they were put in prone position within 48 hours after onset of ALI/ARDS for at least 17 hours per day lasting 7 days. Combining prone position, HFOV or inhaled nitric oxide (INO) did not improve outcome and is only recommended for rescue situations [107].

In pediatric patients, Numa et al. demonstrated that prone position increases functional residual capacity, improving lung compliance and oxygenation [108]. Many clinical case series have shown that prone position improves oxygenation in children [108-118]. Oxygenation improves within a short period (1-2 hours) after position change and can be sustained. For example, Casado-Flores et al. showed in a prospective case study that repetitive changes of positioning every 8 hours improved oxygenation in 18 of 23 children with ARDS; however, mortality was not affected [113]. The largest prospective multi-center RCT did not find any differences between both groups on important outcome variables such as ventilator-free days and mortality [111]. Despite these disappointing findings, it has been shown that regular prone position is able to ameliorate the degree of VILI [110;119-121]. In summary, at the moment there is not enough evidence to recommend prone position for routine use. However, "turning the child round" in a more favorable position/condition should not be disregarded based on all the physiological/experimental rationales that have been provided [106;110;122]

Selective pulmonary vasodilation

Inhaled nitric oxide

Hypoxemia in ALI is mainly caused by ventilation/perfusion mismatch with increased intrapulmonary shunting due to dysregulation characterized by pulmonary vasodilation in non-ventilated (hypoxic) lung regions and vasoconstriction in ventilated areas as well as pulmonary hypertension. INO has shown to be an ideal selective pulmonary vasodilator improving oxygenation and decreasing pulmonary artery pressure [123-127].

In neonates with persistent pulmonary hypertension, INO minimized the degree of respiratory failure and reduced the need for the extracorporeal membrane oxygenation (ECMO) [128]. In how far INO may reduce chronic lung disease and mortality in preterm neonates is still a matter of debate [129;130].

About 60%-80% of adult patients with ALI/ARDS improved in oxygenation with dosages between 2-80 ppm INO. However this effect was only transient, lasting 24-72 hours [127;131-137].

Only a small number of clinical studies have examined INO in children [138;139]. One RCT found that oxygenation was improved only in children with severe hypoxemia (oxygenation index > 25); however this effect was short-lived [140]. In combination with HFOV further improvement was achieved due to better lung recruitment [141]. Despite these effects, mortality was not reduced. Therefore, after a systematic review and a meta-analysis the conclusion was not to recommend its use routinely because final outcome has not been improved [135;136]. In a European Consensus Conference, leading experts in this field subscribed to this view [142].

Aerosolized prostacyclins

The indications for use of aerosolized prostacyclins (e.g., epoprostenol, iloprost) in ALI/ARDS parallel the indications for INO, i.e. primary and secondary pulmonary hypertension. Inhaled prostacyclin (PGI_2) can be applied easily by means of a standard nebulizer system appropriate for optimal alveolar deposition [143;144]. In 1993, Walrmath et al. showed for the first time that aerosolized PGI_2 selectively lowers pulmonary artery pressure, decreases intrapulmonary shunts, and improves oxygenation in adult patients with ARDS [144].

Only one prospective double-blind RCT examined the effect of PGI_2 in 14 children with ALI. Primary outcome measure was oxygenation. It was found that oxygenation was significantly improved by 26% [143]. The optimal dosage in children for PGI_2 was 30 ng/kg/min and thus higher than the recommended dosage in adult patients of 10 ng/kg/min [145;146]. Mortality was not measured in this study; therefore, based on its level of evidence its use may be only reasonably justified. Regarding costs in a child weighing, for example 18 kg, one hour of PGI_2 therapy of 30 ng/kg/min costs \$12 vs. \$125 for INO (manufacturer's data).

Surfactant

In contrast to neonatal RDS, in ALI there is secondary surfactant depletion and inactivation [6;147]. The best available evidence concerning surfactant treatment in adult patients with ALI/ARDS has been extensively reviewed recently, showing improvement in oxygenation shortly after surfactant instillation; however there was no effect on mortality [148].

In the only well-designed multi-center double-blind RCT on surfactant treatment in children with ALI, the primary outcome measure, ventilator-free days, did not differ between the surfactant and placebo group [149]. Mortality, the secondary outcome measure, was reduced in surfactant-treated children 19% vs. 36%. Interestingly, a novel surfactant preparation (Calfactant, 2 doses of 80 mL/m² administered 12 hour apart) with a high proportion of hydrophobic surfactant associated protein B (SP-B) was used, which seems to be equal to natural surfactant and may resist inhibitory proteins [147]. However, it is too early to draw a final conclusion based on these results due to an uneven distribution of the number of immunocompromised patients in trial groups and an insufficient number of patients for subgroup analysis. Future trials should be sufficiently powered, should use high and repetitive dosages of natural surfactant preparations with sufficient SP-B fraction, and should stratify for relevant patient subgroups (e.g. indirect vs. direct lung injury).

Corticosteroids

It was found that patients with ALI who exhibit early fibroproliferation are at greater risk to die [27]. Therefore, intravenous corticosteroid therapy has been considered the most appropriate target pharmacotherapy for many years [150]. However, several clinical trials of high-dose corticosteroids during the early phase of ARDS failed to show improvements in survival [150-153]. In a large multi-center double-blind RCT a moderate dose of corticosteroids was delivered to patients with persistent ARDS for minimal 7 days [154]. Mortality did not improve at day 60 of follow-up and in a subgroup of patients in whom corticosteroids were started on day 14 after onset of ALI/ARDS mortality at day 180 was even higher. This observation raised serious concerns about the appropriateness of corticosteroid therapy in ALI/ARDS. It was speculated that the pro-inflammatory and anti-inflammatory processes during ALI/ARDS do not occur at similar time points, and that the considerable genetic differences in each individual patient may also play a role [155]. To our knowledge, no RCT addressing corticosteroid therapy in the pediatric age group has yet been published.

Extracorporeal Membrane Oxygenation

There are no RCTs exploring the benefits of ECMO in children with ALI. In a retrospective analysis of children with severe pediatric AHRF (including ARDS patients only as a subgroup) with a predicted mortality of 50% to 75%, ECMO did reduce mortality [156;157].

ECMO can be a life-saving tool in critically ill children suffering from transient illness and who otherwise would die (e.g. during meningococcal septic shock and AHRF) [158].

FUTURE DIRECTIONS

Future research should identify patients at risk for ALI and for VILI, by the discovery of new gene candidates or by identifying clinically relevant risk factors. Clinical trials should attempt to include patients as soon as ALI and VILI develops in order to define new treatment strategies preventing VILI and deterioration of ALI [45]. Furthermore, studies should differentiate between patients with direct or indirect lung injury and between adults and children [60].

Distinct gene candidates have been identified which are associated with lung injury induced by overdistention as a possible cause for VILI [159]. As one of the novel mechanisms, Woesten et al. found increased ACE activity in bronchoalveolar lavage fluid (BALF) of mechanically ventilated rats triggering inflammation and apoptosis within hours. Pre-treatment with an ACE inhibitor reduced ACE activity in BALF, pulmonary inflammation and apoptosis (personal communication, unpublished observation).

Differences in lung damage between children and adults should be addressed. Kornecki et al. found that the still-growing lung of animals had more capacity to recover and to compensate early lung damage and seems therefore less vulnerable to VILI than the fully grown adult lung [160].

New treatment options such as corticosteroids, surfactant and lung protective ventilation should be examined in relation to the underlying diseases. Despite the overwhelming influence of underlying disease on outcome (conditions with immuno-

compromise), novel therapeutic approaches may nevertheless improve outcome of children with ALI (e.g. the ARDS Network study protective ventilation strategy) [89]. Interestingly, in the study of Willson et al., surfactant improved outcome in the subgroup of immunocompromised children (50% vs. 60% mortality) [149]. For this subgroup (80%-100% mortality), DiCarlo et al. reported in a small case study that hemofiltration decreased mortality, when started from early onset of ARDS due to improved fluid management and removal of inflammatory mediators [161]. Larger series should confirm these findings.

Interventions in alveolar coagulation/fibrinolysis might offer new therapeutic options. The interplay between inflammation and coagulation/fibrinolysis contributes significantly to the pathophysiology of ALI [17;162]. Alveolar fibrin turnover is disturbed and misbalanced towards an anti-fibrinolytic intraalveolar milieu dominated by PAI-1 [18;19;21;163-166]. Fibrin depositions trigger *de novo* inflammation and pulmonary fibrosis. Many coagulation inhibitors have been tested to rebalance fibrin turnover including heparin, antithrombin, tissue factor pathway inhibitor, factor VIIa, activated protein C, and thrombomodulin in animal models and/or humans with either sepsis or ALI [21;165]. So far, none of these has achieved clinical approval. From the successful experience in the treatment of sepsis with activated protein C (APC), a promising pilot study in human volunteers after endotoxin administration demonstrated the potential role of APC in ALI [167;168].

Due to the fact that VILI resembles original lung injury in many ways, it was hypothesized that disturbed fibrin turnover might play a role in VILI, preventing resolution of alveolar fibrin depositions. First experimental evidence suggests that traumatic mechanical ventilator settings may suppress alveolar fibrinolysis [169;170]. This observation is important, because high levels of PAI-1 in pulmonary edema fluid (antifibrinolytic alveolar milieu) correlate with mortality [171]. Interestingly, lung protective ventilation attenuates intraalveolar fibrin formation [172]. These preliminary results should encourage investigating the underlying pathomechanisms and novel treatment options.

We need more follow-up data on pediatric ALI. In contrast to the scarcity of pediatric data, follow-up studies in adults showed that a considerable percentage of patients suffer lifelong sequelae (e.g. health-related quality of life, neuro-cognitive dysfunction, and abnormal pulmonary function testing) [40;173-186]. The severity of lung injury, direct vs. indirect lung injury and the duration of mechanical ventilation correlate with persistent abnormalities of pulmonary function.

For the pediatric age groups, it is possible that ALI may interfere with normal lung development thus causing chronic lung disease [187]. A few small-sized observational cohort studies have reported respiratory sequelae of 33%-100%. [188-192]. Different kinds of respiratory function abnormalities have been measured and most of them are subclinical: obstructive and restrictive functional abnormalities. For example, an observational follow-up study of pre-school children (mean age 37 months) after a period of septic shock and ARDS found that 2 of 7 children suffered from relevant respiratory sequelae [192]. Three months after discharge almost all children experienced restrictive and/or obstructive abnormalities and recovery during the following months reached plateau levels at the 12-month visit with no further improvement [40;188;193]. Recovery is however limited to a period of 6-12 months [40;193]. This shows that the timing of the follow-up examination is crucial and mainly determines the incidence of abnormalities (Figure 6).

No specific therapy for respiratory function abnormalities exists. Inhalation therapy with beta-2 adrenergic bronchodilators for obstructive lung disease has been tried; however, neither the patient's condition nor the respiratory function tests were improved [188;192].

The positive effects of lung protective ventilation strategies on prevention of respiratory function abnormalities and long-term sequelae need to be determined [89;194].

Figure 6. Number of patients with recovery within the first 3 months and 12 months after acute respiratory distress syndrome (data from reference (41)).

CONCLUDING REMARKS

Future research should concentrate on the pediatric age group, the complex and heterogeneous pathophysiology (e.g. fibrinolysis), the repair mechanisms and the genetic and gender-related conditions. [73;170]. Furthermore, pediatric critical care physicians should be aware of and search for the short-term and long-term sequelae of pediatric ALI. Whether lung growth will put the child in a more favorable situation compared with an adult (compensation of lung damage by growth?) also needs to be answered. Therefore, a concerted effort is still needed to address all these factors related to pediatric ALI.

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OUTLINE OF THE THESIS

Chapter I presents a definition of acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) and outlines current knowledge on the pathophysiology, epidemiology and treatment. Further, we summarize the most important evidence emerging from clinical studies on pediatric ALI.

Aims of the study

There is very limited clinical research concerning pediatric ALI. Therefore, the main objective of this thesis was to gather new data on pediatric ALI. We started with clinical studies and we performed experimental studies in a new area, i.e. the relationship between mechanical ventilation and alveolar fibrinolysis.

Chapter III presents two epidemiological studies. First, we determined the incidence and outcome in children admitted to a large multi-disciplinary pediatric intensive care unit over a two-year period. For each child the underlying disease was established and the risk factors were calculated. Second, we analysed the data of a large multinational European database on pediatric ARDS with respect to differences related to gender. Recent observations have indicated that gender may determine the incidence and outcome of patients with critical diseases such as sepsis and ALI/ARDS.

In **Chapter IV** we conducted a randomized controlled trial with aerosolized prostacyclin to investigate the effects of selective pulmonary vasodilation on the extent of oxygen uptake. During ALI, any gain in oxygenation may contribute to an overall improvement in outcome, and the combination of different selective pulmonary vasodilators may enhance this effect.

In **Chapter V**, due to new insights into the inter-relationship between pulmonary inflammation and intraalveolar coagulation, we hypothesised that mechanical ventilation may affect alveolar fibrinolysis. Persistence of alveolar fibrin contributes to surfactant inactivation and promotes alveolar fibrosis. We performed two experiments in two different models of lung injury; firstly in healthy rat lungs and secondly in pre-injured lungs (lipopolysaccharide-induced lung injury).

There are very few data on the follow-up in children with ALI. In **Chapter VI** we postulated that the smaller the child the greater the impact of ALI and mechanical ventilation on pulmonary function. This was based on the fact that in infants and pre-school children the lung is still growing and developing. Therefore, we measured the respiratory sequelae in children with meningococcal septic shock and ALI/ARDS one year after their discharge from hospital.

Chapter VII presents a summary of the main findings of this thesis and briefly discusses suggestions for future research.

EPIDEMIOLOGY

Part A

Incidence and short-term outcome of acute lung injury in mechanically ventilated children

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ABSTRACT

The aim of this study was to determine the incidence and short-term outcome of mechanically ventilated children suffering from acute lung injury (ALI) on a pediatric intensive care unit (PICU). Between January 1 1998 and January 1 2000, all mechanically ventilated children were evaluated using the criteria of an American-European Consensus Conference. Of the 443 children eligible for analysis, 44 (9.9%) were diagnosed as suffering from ALI. Of these, 79.5% developed the acute respiratory distress syndrome (ARDS); 54.5% (24 of 44) fulfilled the ARDS criteria at inclusion and 25% (11 of 44) later. PICU mortality for ALI was 27.3% (12 of 44) and within the ARDS subgroup 31.4% (11 of 35). Of the 12 children who died, 11 had ARDS; the main cause of death was cerebral damage (seven of 12). ALI and ARDS are rare diseases on a pediatric intensive care unit with a high mortality. Most of the children with ALI develop ARDS. In the ARDS subgroup, mortality is higher than in the ALI non-ARDS subgroup. Further investigations should confirm prognostic factors (e.g. respiratory parameters) for prediction of outcome.

INTRODUCTION

In 1994, an American-European Consensus Conference (AECC) defined the criteria for what has been known as acute respiratory distress syndrome (ARDS) [1] and was first described by Ashbaugh et al. in 1968 [2]. This consensus was necessary because in the past heterogeneous criteria for ARDS were used to include patients in clinical studies, thus precluding comparative studies and definite conclusions. The intention of the AECC was to find a uniform definition to provide more homogeneity and comparability for future research in this field. As a result, acute lung injury (ALI) was introduced as a new term for this disease. This partly replaced the old term ARDS because ALI was considered to more accurately reflect latest insights on the pathophysiological process of this disease. Since then, the term ARDS has been reserved for the most severe form of ALI.

Pathophysiologically, ALI is considered to be an acute inflammatory reaction of the lung with damage to the epithelial-endothelial barrier, causing high permeability pulmonary oedema. Lung compliance is decreased whereas the ventilation/perfu-

sion mismatch increases, resulting in failure of gas exchange. Different intrapulmonary aetiologies, such as pneumonia and aspiration (direct lung injury) and extrapulmonary aetiologies, such as septicaemia and multitrauma (indirect lung injury), may trigger this process.

Since the AECC, studies in adult patients have provided data on the incidence and outcomes of ALI/ARDS [3–5]. No studies have specifically investigated the incidence and outcomes of ALI in children. Only two studies, one investigating the ARDS subgroup, and another using a broader definition of acute hypoxic respiratory failure (AHRF), have so far published data on respiratory failure in children. The former was performed on a pediatric intensive care unit (PICU) in Kuala Lumpur, Malaysia [6], and the second one on a PICU in London, UK [7]. Furthermore, the European ARDS network collects epidemiological data on pediatric ARDS from multiple European centres, which are accessible on the internet [8]. Due to the lack of specific data on pediatric ALI, the authors aimed to determine the incidence and short-term outcome of ALI in mechanically ventilated children.

METHODS

After approval from the local Ethics Committee, all admissions from January 1 1998 to January 1 2000 were analysed. The PICU is a 12-bed, multidisciplinary, tertiary referral centre and subdivision of the Emma Children's Hospital, Academic Medical Centre of the University of Amsterdam. Children with all types of diseases are admitted based on the following criteria: impending or manifest organ failure of at least one vital organ (respiration, circulation or neurology), patients with a high risk of organ dysfunction and failure due to general pediatric surgery (including multiply injured or neonatal surgical patients) or postorthopaedic surgery, acute peritoneal dialysis or haemodialysis, postcardiac surgery, postneurosurgery and patients whose organ function needs to be closely monitored independent of the underlying disease. The age group of the admitted children ranges from newborns with a birth weight of > 2 kg (independent of postconceptional age) to adolescents with a maximum age of 18 years. Figure 1 presents an overview of the study population (1,100 children) according to the discipline involved.

For the purpose of the study, only mechanically ventilated children who survived the first 24 h of admission were included. Death and disturbances in gas exchange in the first 24 h are frequently caused by situations not related to ALI, such as failed resuscitation in septic shock or cerebral herniation (e.g. neurotrauma, cerebral tumours). Furthermore, during this period children are often haemodynamically instable and prone to multiple interventions, such as fluid resuscitation or intubation with aggressive sedation and relaxation. These interventions can aggravate hypoxaemia not related to the pathophysiology of ALI and can therefore lead to a false-positive high incidence of ALI. Post hoc, children who died during the first 24 h were analysed to determine if they would have fulfilled the AECC criteria in order to assess the degree of underestimation of incidence and mortality of ALI due to this 24-h criterion.

The ventilation strategy of the unit is to ventilate children with ALI in the pressure-controlled mode, with positive end-expiratory pressure (PEEP) titrated based on oxygenation and positive inspiratory pressure (PIP), whenever possible limited to a maximum of 30 cmH₂O with permissive hypercapnia if required. When there is evidence of lung derecruitment, recruitment manoeuvres (e.g. prone positioning, changes in ventilator settings) are performed to improve oxygenation.

All ventilated patients were analysed on the second day of admission, whether or not they fulfilled the AECC criteria for ALI: 1) acute onset; 2) arterial oxygen tension to fraction of inspired oxygen ratio ($\text{PaO}_2/\text{FIO}_2 \leq 40 \text{ kPa}$) for ALI and $\text{PaO}_2/\text{FIO}_2 \leq 26.7 \text{ kPa}$ for ARDS; 3) no remaining clinical signs of atrial hypertension; and 4) bilateral infiltrates on chest radiographs. For the purpose of the study, criteria 2 and 3 were further elaborated. Firstly, only patients whose $\text{PaO}_2/\text{FIO}_2$ was $\leq 40 \text{ kPa}$ in two consecutive measurements with a minimum interval of 8 h were included. Average $\text{PaO}_2/\text{FIO}_2$ was then calculated for each day from inclusion to extubation or death, based on multiple daily blood-gas analysis (at least every 8 h) and the recorded ventilator settings. Secondly, it was diagnosed whether or not left atrial hypertension was clinically present, as suggested by the AECC [1]. As the gold standard of measuring left atrial hypertension with a pulmonary artery catheter is not routinely used in children, cardiac echography was performed when there was doubt about the

left ventricular function or when the patients were receiving vasopressor agents. This was performed with the aim to exclude patients with a high risk of cardiogenic pulmonary oedema based on the following criteria: decreased shortening fraction of $<30\%$, mitral regurgitation and/or enlarged left atrium. The method and the criteria have been described in detail elsewhere [9,10]. A pediatric radiologist and a pediatric intensivist, both blinded for clinical information, analysed the chest radiographs for the presence of bilateral infiltrates consistent with lung oedema and interobserver variation was determined.

All patient data (e.g. ventilation methods and requirements, biochemical and physiological variables) were collected prospectively and documented on the charts. The unit policy is to evaluate the diagnosis of ALI in all ventilated children when the $\text{PaO}_2/\text{FIO}_2 \leq 40$ kPa. This meant that chest radiographs were performed, if these were not made within the previous 4 h. Furthermore, the hourly documented ventilator settings were linked simultaneously with the blood-gas analysis. As part of the routine, the attending physician also evaluated the haemodynamic condition of the patient and indicated echocardiography, if needed. All these data were documented in the patients' charts and were accessible for analysis. Additionally, a differentiation was made between direct (e.g. pneumonia, aspiration) and indirect (e.g. sepsis, multitrauma, postsurgical) lung injury. The outcome parameter was PICU mortality. The cause of death was recorded as failed resuscitation, untreatable circulatory, respiratory or neurological failure (brain death or severe brain damage) with or without withdrawal of therapy due to a poor prognosis.

For the estimation of severity of disease and for comparison between survivors and nonsurvivors the pediatric index of mortality (PIM) [11], the pediatric risk of mortality score (PRISM) II [12] and a maximum score for multiorgan system failure (MOSF) during hospitalisation were assessed [13]. In addition to the well-developed indices for mortality prediction at admission (i.e. PIM) or 24 h after admission (i.e. PRISM II), respiratory parameters (e.g. $\text{PaO}_2/\text{FIO}_2$) shortly after diagnosis of ALI were statistically analysed to determine whether they independently correlated with mortality.

Statistical analysis

Cohen’s kappa was calculated to determine interobserver variation between the pediatric intensivist and radiologist. Collected data on survivors and nonsurvivors were compared. One-sided t-tests (unpaired), Chi-squared tests, Fischer’s exact tests, or Mann Whitney U-tests were used based on the measurement scale and the exploration of distributional characteristics. Significant parameters from the univariate analyses and predictors for mortality (PIM, PRISM II) were selected for multivariate binary logistic regression analysis against survival.

Figure 1. Profile of intensive care unit population (n = 1,100) admitted during the study period.

Table 1. Patients' characteristics

	All	Survivors	Nonsurvivors	Test value
Subjects n	44	31	13	
Age months	27.5 (5.0-58.3)	26.0 (3.5-55.8)	36.0 (9.0-134.8)	Z=-1.19
Weight kg	13.5 (6.3-20.0)	12.5 (6.3-20.0)	17.5 (7.5-43.0)	Z=-1.10
Male	24 (54.5)	17 (53.1)	7 (58.3)	Chi ² =0.10
PIM	-2.393±1.189	-2.519±1.111	-2.056±1.373	t=-1.154
PRISM	16.7±9.6	14.6±8.7	22.3±10.1	t=-2.50*
Multiorgan system failure	29 (65.9)	17 (53.1)	12 (100.0)	Chi ² =8.53*
Direct lung injury	21 (47.7)	15 (46.9)	6 (50.0)	Chi ² =0.04
Disease, meningococcus infection or septic shock	15 (34.1)	13 (40.6)	2 (16.7)	Chi ² =2.23
ARDS at inclusion	24 (54.5)	15 (46.9)	9 (75.0)	Chi ² =2.78 [†]
PaO ₂ /FIO ₂ day 1 after inclusion kPa	28.2±13.8	30.5±11.9	22.1±17.2	t=1.84*
PaO ₂ /FIO ₂ day 2 after inclusion kPa	27.8±13.6	28.4±10.9	26.4±19.7	t=0.43*
PIPmax cmH ₂ O	33.1±7.2	31.7±6.9	37.0±6.6	t=-2.30*
PEEPmax cmH ₂ O	9.5±4.1	8.8±3.9	11.4±4.2	t=-1.96*
Ventilator days	6.5 (5.3-13.0)	7.0 (6.0-13.0)	4.0 (2.0-8.0)	Z=-2.13 [#]

Data are presented as median (interquartile range), n (%) or mean ± SD, unless otherwise stated. PIM: pediatric index of mortality; PRISM: pediatric risk of mortality; ARDS: acute respiratory distress syndrome; PaO₂/FIO₂: average arterial oxygen tension to fraction of inspired oxygen ratio; PIPmax: positive inspiratory pressure; PEEPmax: positive end-expiratory pressure. The Z-value is derived from Mann Whitney U-tests and the t-tests were unpaired.

*: p<0.05 one-sided; [†]: p<0.05 two-sided; [#]: p=0.05-0.10 one-sided.

Table 2. Data on multivariate regression analysis

Factor	β	se	Exp(β)	95% CI	p-value
PRISM	0.097	0.04	1.101	1.019-1.190	0.015
PaO ₂ /FIO ₂ day 1 after inclusion	-0.099	0.03	0.906	0.854-0.961	0.001

CI: confidence interval; PRISM: pediatric risk of mortality; PaO₂/FIO₂: arterial oxygen tension to fraction of inspired oxygen ratio (average score on day 1 after inclusion). McFadden's-R²= 33.4%. Overall % correctly predicted = 86.4. Area under the curve (combination of PaO₂/FIO₂ on day one after inclusion and PRISM): 0.815 (95% CI: 0.642-0.989).

RESULTS

Incidence

In the 2-year study period, 446 of the admitted children were mechanically ventilated. The clinical or radiographical status of three patients was not retrievable, and thus 443 were eligible for analysis. The interobserver agreement between the pediatric intensivist and radiologist with respect to judgement of the chest radiographs was excellent (Cohen's kappa of 0.772). All four AECC criteria for ALI were fulfilled by 44 children (9.9%). Of these 44 children, 41 were included on admission day 2, and three on day 3; all of them were mechanically ventilated on the first day of admission. In a subgroup analysis, 79.5% of the ALI patients (35 of 44) were diagnosed as suffering from ARDS. Of these, 54.5% (24 of 44) immediately fulfilled the ARDS criteria at inclusion and 25% (11 of 44) developed ARDS later on in the course of their disease. The incidence of ARDS among all mechanically ventilated patients was therefore 7.9%. This subgroup analysis is shown in Figure 2 and the patients' characteristics are presented in Table 1.

Post hoc, all PICU deaths (36 of 70, 51.4%) during the first 24 h were analysed to establish whether they would have been diagnosed as ALI and may have been missed for this study purpose. None of them fulfilled the AECC criteria for ALI, mostly due to cardiac failure.

Of the underlying diseases, meningococcal septic shock (indirect lung injury) was the most frequent (34.1%) followed by aetiologies with direct lung injury, respiratory syncytial virus infection (15.9%) and primary pneumonia (11.4%). Four patients (9.1%), including those with oncological diseases, were immunocompromised. Twenty-nine children had accompanying MOSF (65.9%).

Eight children developed a pneumothorax as a complication; all had ARDS and four of them died. Nine children (all had ARDS) needed one or more of the following specialised intensive care treatments due to intractable respiratory failure: inhaled nitric oxide (nine cases, four died), high frequency oscillatory ventilation (two cases, one died) and extracorporeal membrane oxygenation (one case).

Mortality

Twelve of the 44 patients died (27.3%) and of these 11 had ARDS. The mortality rates in the different subgroups are shown in Figure 3. All except three patients died during the first 8 days after the onset of ALI. In seven patients, the cause of death was severe irreversible cerebral damage, which was caused by the initial insult (i.e. shock, near drowning, smoke inhalation, strangulation). Of the remaining five children, two died due to intractable circulatory failure (septic shock), two (4.5% of ALI) due to intractable respiratory failure (pulmonary haemorrhage, both had a congenital immune disorder) and one died due to veno-occlusive disease. Of the four patients, who were immunocompromised, two died.

Figure 2. Incidence of acute lung injury (ALI) in the subgroups: acute respiratory distress syndrome (ARDS) and ALI non-ARDS.

Figure 3. Mortality rates of acute lung injury (ALI) and in the subgroups: acute respiratory distress syndrome (ARDS) and ALI non-ARDS.

Table 1 presents the results of the univariate analysis comparing nonsurvivors with survivors. Significant differences for nonsurvivors and survivors were found for the PRISM, MOSF, $\text{PaO}_2/\text{FIO}_2$ on day 1 after inclusion, maximal PIP and maximal PEEP. Due to the small number of patients ($n=44$) and to prevent multicollinearity, only three parameters were analysed from this set in the “best” multivariate binary logistic regression model: PIM, PRISM and $\text{PaO}_2/\text{FIO}_2$ on day 1 after inclusion. The $\text{PaO}_2/\text{FIO}_2$ on day 1 after inclusion and PRISM II were associated with mortality with an area under the receiver operating characteristic curve (AUC) of 0.728 and 0.732, respectively (Table 2). Combining both, the AUC improved to 0.815 compared with each parameter alone.

DISCUSSION

To the best of the authors’ knowledge, this is the first report specifically detailing the incidence, patients’ characteristics and outcome of pediatric ALI in a Western European PICU. The basic findings are: 1) an incidence of 9.9% of ALI of the ventilated children with 80% of them developing ARDS; 2) a higher mortality in the ARDS subgroup compared with the ALI non-ARDS subgroup; and 3) 25% of children with ALI at inclusion deteriorated later to ARDS. As there is a lack of precise data in the literature, it seems appropriate in some cases to make comparisons with surveys that have investigated comparable groups of children with similar pathology (AHRF/ARDS), whose inclusion criteria were also based on the AECC. In case such data were unavailable, comparisons with adult studies have been made.

Only the incidence of ARDS, as the largest subgroup of ALI patients, has been studied so far. In an Asian population an incidence of ARDS of 4.1% was found [6], which was higher than the 3.2% (35 of 1,100) in the present PICU population (Figure 2). This difference may be explained by higher admission thresholds, higher severity scores for nonsurvivors (PRISM 30.4 versus 22.3) and higher incidences of infections (e.g. sepsis 61.5% versus 16.7%) in the Asian study.

A total of 79.5% of the present ALI patients developed ARDS. At inclusion, however, only 54.5% had ARDS and 25% developed ARDS later in the course of their disease. This observation supports one of the intentions of the AECC, whereby ALI was

introduced to allow early diagnosis of the disease for scientific and clinical purposes [1,3,4]. The 25% of patients who deteriorated to ARDS could be an important target population for novel therapies (e.g. protective ventilation strategies, drug therapy) in order to prevent the development of ARDS and to improve outcome [14,15]. Furthermore, the causes of deterioration should be studied in the context of ventilator-induced lung injury (VILI) and MOSF [15,16]. The high percentage (79.5%) of children with ALI, who finally developed ARDS was similar to the data from two large adult studies (76–80%) [3,4].

ALI represents severe respiratory failure and is frequently part of MOSF. The incidence of MOSF in the ALI population (65.9%) was almost twice as high as the 33.9% in a study that used a more general definition of pediatric AHRF [7]. This difference could be explained by the fact that their definition of AHRF also included children without infiltrates on the chest radiograph (criterion 4), resulting in the inclusion of less severely ill children.

As mentioned above, inclusion was started 24 h after admission in order to avoid an overestimation of the incidence of ALI. Furthermore, death shortly after admission may be caused by the initial insult rather than by deteriorating respiratory failure. In order to elucidate a possible bias selection due to this criterion, a post hoc analysis of all PICU deaths (36 of 70, 51.4%) was performed during the first 24 h to establish whether or not they would have been diagnosed as ALI and may have been missed for the analysis. However, none of them fulfilled the AECC criteria for ALI before death. Neither specific mortality data for pediatric ALI nor standardised mortality ratios (SMR; i.e. observed deaths divided by expected deaths) have yet been published. With respect to the subgroup ARDS, however, two European investigations reported crude mortality rates of 32% and 36.5% [7,8], which are similar to the 31.4% found in the present study, and in an Asian population a mortality rate double that found in Europe was reported (62%) [6]. An appropriate interpretation of the differences in mortality rates between these studies can only be made by the comparison of the SMR for each unit. However, published data do not allow the calculation of SMRs. Therefore, it can only be hypothesised that the differences in mortality rates may be caused by differences in patient populations between these studies. For example,

when compared with this study, Goh et al. [6] reported, within the same group of patients (all were mechanically ventilated and fulfilled the AECC criteria for ARDS), more sepsis patients (61.5% versus 16.7%) and higher PRISM scores (30.4 versus 22.3) among the nonsurvivors.

For the western part of the world, mortality in adult and pediatric ARDS patients has been declining in the last decade to 30–40%, which has been attributed to an overall improvement of intensive care treatment and the changes in ventilation strategies [17–21]. When the subgroups within the present ALI population were compared, it was observed that of the 12 children who died, only one of nine was in the ALI non-ARDS group, whereas 11 of 35 were in the ARDS group (Figure 3). This is in contrast to the adult literature, where mortality did not differ between the ALI non-ARDS and the ARDS subgroups [5].

In the present study, the leading cause of death was cerebral damage (seven of 12) suffered as a result of the initial insult. Only two patients died due to pulmonary complications and both had pre-existing complex congenital immune disorders. This suggests that the initial trigger negatively influences further organ dysfunction and final outcome. One pediatric study on AHRF and multiple adult studies showed that underlying diseases, such as cerebral dysfunction and immune disorders, have a greater impact on outcome than respiratory failure alone [7,18,22–28]. These findings suggest that ALI may be more a symptom than a limiting disease. However, this seems to contradict the strong evidence that ongoing pulmonary inflammation during ALI, and secondarily caused by VILI, triggers a *de novo* systemic inflammatory response, leading to multiple organ failure and an increase in mortality [15,16]. Future studies in children should therefore define the clinical situations in which ALI is just a symptom and resolves together with the underlying disease, or a contributing factor for multiorgan failure and negative outcome.

In this context, it would be desirable to have parameters for decision-making at the bedside and for selection of patients for new therapies and clinical trials. Respiratory parameters (e.g. oxygenation indices) may be suitable because they represent pulmonary function and indicate deterioration of ALI, before a systemic response is initiated and outcome negatively affected.

One of the most frequently studied indices is the $\text{PaO}_2/\text{FIO}_2$, although contradictory results concerning its ability to predict outcome have been reported in adult AHRF/ARDS [2,27–35]. Some authors have argued that the ratio may not be predictive at inclusion, but after 24 or 48 h [30,31]. Two pediatric studies on children with generalised AHRF also failed to clearly define respiratory parameters for outcome prediction [6,7].

Due to the small number of children with ALI ($n=44$) in the present study, the number of respiratory parameters for multilogistic regression analysis had to be limited. Therefore, only those parameters that can be early and easily performed at the bedside were focused upon ($\text{PaO}_2/\text{FIO}_2$ on day 1 and 2 after inclusion). The $\text{PaO}_2/\text{FIO}_2$ was compared with two standardised scoring systems for mortality prediction in children (i.e. PIM and PRISM). The PIM is performed at admission and accounts for the pre-existing condition of the patient, whereas the PRISM is performed 24 h after admission and also reflects the progression of disease during the first hours. The analysis revealed that the AUC of the $\text{PaO}_2/\text{FIO}_2$ on day 1 after inclusion was similar to the AUC of the PRISM, and that the combination of both resulted in an improvement of the AUC (0.815). Interestingly, the PIM did not discriminate between survivors and nonsurvivors of ALI. Two possible explanations for the findings were considered. Firstly, the PIM may not be reliable for specific diagnostic subgroups such as ALI, because it was developed for a PICU population as a whole [11]. This explanation is supported by a recent report, where the PIM of children with respiratory failure showed a smaller AUC than the overall PICU population [36]. Secondly, another reason may be that the PIM of the ALI nonsurvivors was very low -2.056 ± 1.373 . This may be explained by the fact that they survived the first 24 h (inclusion criterion for the diagnosis of ALI) and therefore may belong to a group of patients with a lower PIM score compared with other PICU patients, who died during the first 24 h (i.e. early nonsurvivors). Indeed, PICU patients who died during the first 24 h had a significantly higher PIM score of 0.622 ± 2.652 compared with the nonsurvivors with ALI ($p<0.004$). In other words, the late nonsurvivors of the ALI patients had been less severely ill at admission than the early nonsurvivors of the whole PICU population. Obviously the most severely ill children on a PICU die on the

first day and for them PIM is a reliable predictor, whereas for nonsurvivors with ALI, who die later, the PIM loses reliability.

In summary, due to the small number of patients with ALI and the limited number of parameters used for regression analysis, these results only suggest a possible association between a respiratory parameter ($\text{PaO}_2/\text{FIO}_2$) and outcome. Therefore, future studies should confirm this association, considering the vicious circle of lung injury: localised pulmonary inflammation due to an initial trigger (e.g. pneumonia), secondary lung damage due to mechanical ventilation, and finally the development of a systemic inflammatory response negatively influencing outcome.

To conclude, ALI and ARDS are uncommon diseases in a pediatric intensive care unit but have a high mortality. Approximately 80% of children with ALI develop ARDS. The mortality in the ARDS subgroup is higher than in the ALI non-ARDS subgroup and is determined by the underlying diseases. Future investigations should confirm what factors indicate the deterioration from ALI to ARDS and predict outcome. Children who are at risk can then be identified and may benefit from novel therapeutic approaches.

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EPIDEMIOLOGY

Part B

Gender-based differences in children with sepsis and ARDS: The ESPNIC ARDS Database Group

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ABSTRACT

Male gender predisposes to severe sepsis and septic shock. This effect has been ascribed to higher levels of testosterone. The ESPNIC ARDS database was searched, to determine if there was evidence of a similar male preponderance in severe sepsis in prepubertal patients in spite of low levels of male sex hormones at this age. A total of 72 patients beyond neonatal age up to 8 years of age with sepsis were identified. The male/female (M/F) ratio was 1.7 (1.0;2.7) and differed significantly from non-septic ARDS patients in this age group [$n = 209$; M/F = 1.0 (0.8;1.3)]. The highest M/F ratio was observed in the first year of life. The gender-ratio was the same as reported in adult patients with sepsis. In infants between 1 month and 12 months of age, the ratio was 2.8 (1.2;6.1) ($\text{Chi}^2 = 5.6$; $P < 0.01$), in children from 1 year to 8 years of age it was 1.2 (0.7;2.2) (n.s.). In a subgroup of patients with severe sepsis or septic shock, caused by other bacteria than *Neisseria meningitidis*, the M/F ratio was 2.1 (1.2;3.6) ($\text{Chi}^2 = 4.9$; $P < 0.05$), while in patients with meningococcal sepsis ($n=20$) the M/F ratio was 1.0 (0.4;2.3). In pre-pubertal ARDS patients with sepsis an increased frequency of male patients is found, comparable to adults. No male preponderance exists in patients with ARDS due to meningococcal septic shock. Since levels of testosterone and other sex hormones are extremely low at this age, we conclude that factors other than testosterone are involved in the male preponderance in severe sepsis.

BACKGROUND

A high proportion of male patients among surgical patients with sepsis was first reported by McGowan in 1975 [1]. Male gender as a risk factor for the development of severe sepsis and septic shock has been highlighted recently [2,3,4]. The interaction between gender and the occurrence of critical illness in children is not new. An excess morbidity of boys has been described in upper respiratory infection, bronchiolitis, pneumonia, and septicaemia [5]. The influence of sex hormones on the host immune response has been proposed as an explanation for this unequal gender distribution in sepsis [3,6,7,8]. To investigate the influence of gender on the occurrence of severe sepsis and septic shock we analysed data of the ESPNIC ARDS database

(www.meb.uni-bonn.de/ards). This database, originating from an informal German working group on pediatric Acute Respiratory Distress Syndrome (ARDS) and endorsed by the European Society of Pediatric and Neonatal Intensive Care (ESPNIC) since 2000, has compiled clinical data on around 400 cases of pediatric ARDS since 1991. The data collection has been performed retrospectively and prospectively. All patients fulfil the definition criteria of ARDS according to the consensus conference [9]. Data have been made anonymous in accordance with European regulations [10]. To test the hypothesis that higher levels of testosterone or other sex hormones are responsible for male preponderance in severe sepsis and septic shock, the database was searched for patients without sepsis before puberty, in whom sex hormone levels are low.

METHODS

The “ARDS Database” was searched for all prepubertal patients with a diagnosis of sepsis or septic shock, treated from 1 January 1991 through 31 December 2001. Precocious puberty is defined by the onset before the age of 8 years in females and 9 years in males. To avoid statistical bias, male patients between 8 years and 9 years of age were omitted. Patients with X-linked immunodeficiency (X-linked lymphoproliferative disease, Wiskott-Aldrich syndrome) were excluded. The database does not include any term neonates or ex-premature patients younger than 43 gestational weeks. Patient demographics and underlying diseases are given in Table 1. All patients fulfilled the criteria of severe sepsis or septic shock according to the ACCP/SCCM consensus conference, defining it as a systemic inflammatory syndrome (SIRS) in response to infection that is associated with acute organ dysfunction [11]. Descriptive statistics (frequency, 95% confidence interval) of the sex ratio were applied to the group of patients with severe sepsis and/or septic shock as a whole, and to subgroups according to the causative agent, to chronic underlying diseases, and to age.

The frequency of male gender in the groups of patients with sepsis was compared to the frequency among ARDS patients of the same age group without sepsis, using the chi-square test where appropriate.

Table 1. Comparison of the gender ratio (M/F ratio) between prepubertal ARDS patients with and without sepsis

Acute causative disease	Male	Female	M/F ratio	X ²
Other than sepsis	105	104	1.0(0.8;1.3)	0.0; ns.
Sepsis (total)	45	27	1.7(1.0;2.7)	3.2;p<0.05
Causative organism				
<i>N. meningitides</i>	10	10	1.0(0.4;2.3)	0.00; n.s.
Other gram-negative	7	4	1.8(0.5;5.6)	n.a.
Gram-positive	7	2	3.5(0.8;15)	n.a.
Fungi, and not specified	21	11	1.9(0.9;3.9)	2.6; n.s.
Organisms except <i>N. meningitides</i> (total)	35	17	2.1(1.2;3.6)	4.9;0<0.05
Age	Male	Female	M/F ratio	X ²
1-12 months	22	8	2.8(1.2;6.1)	5.6; p<0.01
>12-96 months	23	19	1.2(0.7;2.2)	0.3; n.s.
Underlying diseases	Male	Female	M/F ratio	X ²
None	22	15	1.5(0.8;2.8)	1.1; n.s.
Immunocompromising disease	15	9	1.7(0.7;3.7)	1.3; n.s.
Other chronic disease	8	3	2.7(0.8;9.3)	n.a.

Figure 1. Gender distribution (male/female ratio) in prepubertal patients with ARDS with and without sepsis.

RESULTS

Information on patient gender was available in 384/391 cases registered from January 1991 to December 2001. Five patients suffered from X-linked immunologic disorders (Wiskott-Aldrich Syndrome, septic granulomatosis, X-linked lymphoproliferative disease). Excluding patients older than 8 years and patients with X-linked chronic underlying disease, 281 cases were eligible for analysis (Figure 1). Seventy-two patients had a diagnosis of sepsis with an overall mortality of 43.6%. The highest mortality (83.3%) was observed in immunocompromised patients, in immunocompetent patients it was 24%. Overall mortality declined from 58.3% before 1996 to 37.5% in later years. Mortality was 40% in boys and 53% in girls.

The descriptive statistics of the groups of patients are given in Table 1. Compared to patients without a diagnosis of sepsis, frequency of male gender was increased in the total group of patients with severe sepsis or septic shock (Table 1). Patients in various subgroups were not equally affected by an increased M/F-ratio. No male preponderance was found in a subgroup of meningococcal septic shock and it was not significant in children after infancy.

DISCUSSION

Our data show that the increased prevalence of male gender in patients with severe sepsis and septic shock exists before the onset of puberty. There is no difference to the sex ratio observed in adults. Wichmann [3] reports a M/F ratio of 1.86 in adult surgical ICU patients with an odds ratio for the development of septic shock of 1.5. The proportion of male patients in children with sepsis with ARDS is even higher than recently reported by Watson et al. [12], who found a M/F ratio of 1.22 and 1.33 in infants and in children from 1 year to 9 years of age, respectively. This study analysed data of 9,675 patients from the USA, including neonates, who were treated in 1995 and had a diagnosis of sepsis with failure of one or more organ systems. A low case fatality rate of 10% shows that the average severity of disease in this analysis was lower than in patients of the ARDS database.

We found only a moderate increase in the M/F ratio, comparable to the observation of Watson et al. [12] in children beyond infancy, while infants from 1 month to 12

months of age showed marked male preponderance. The difference between both age groups cannot be explained by the influence of sex hormones. No increased frequency of male gender was observed among patients with sepsis due to *N. meningitidis*. Seventy-five percent of these patients were older than 1 year. This may contribute to the unequal M/F ratio in the two age groups. Unfortunately, the low number of patients and the lack of information about the causative organism in nearly half of the patients made multivariate statistical analysis impossible.

The only subgroup caused by a single organism, large enough to allow statistical testing, were patients with meningococcal sepsis. An equal gender distribution in meningococcal septic shock was also described in the patient characteristics of the rBPI21-study [13] and observed in the patients investigated for PAI-1-gene polymorphism (W. Zenz, personal communication). Patients with a severe course of meningococcal septic shock differ from others by the presence of purpura fulminans. Arterial thrombotic occlusion is pivotal for the clinical course in purpura fulminans and individual characteristics of the coagulation system predispose to a more severe course. Geishofer et al. [14] described an increased frequency of the 4G/4G polymorphism of the promoter region of the plasminogen activator inhibitor 1 (PAI-1) gene in patients with severe meningococcal sepsis, especially in patients with ARDS. This polymorphism correlates with a more severe course of the disease. PAI-1 is inherited autosomally. This may explain why no male preponderance was observed in patients with meningococcal septic shock.

Male preponderance was more pronounced in patients with sepsis-induced ARDS, due to other bacteria than *N. meningitidis*. The high proportion of male individuals is not explained by an elevated M/F ratio in patients at risk. Severe sepsis and septic shock due to other organisms than *N. meningitidis* as the cause of pediatric ARDS mostly occurred in patients with an immunocompromising disease, which in the majority resulted from malignant diseases or in patients with other chronic underlying disease. It is a major disadvantage of this type of study that no exact denominator in terms of patients with chronic disease in the total population or total number of those patients treated at the participating institutions is available. It is known, however, that the M/F ratio in pediatric patients with malignant disease

is below 1.2/1 [15]. Patients with X-linked immunocompromising disease were excluded from our analysis. Selection bias could arise from the fact that critical respiratory illness in children predominantly affects male patients [5]. However, the M/F ratio was only 1.12 in patients in whom sepsis and ARDS were secondary to pneumonia, and no male preponderance was found in non-septic pediatric ARDS cases. Selection bias might also result from male preponderance in immunocompetent patients with chronic disease. In these patients the need of invasive procedures or an impaired inability to clear secretions eventually increases the risk of sepsis. However, the M/F ratio in infants with ARDS without sepsis was 1.35, while the M/F ratio in patients with sepsis of the same age group was 2.7. From this we conclude that the observed gender difference is not explained by selection bias. Testosterone levels in prepubertal children are extremely low and are below the levels in adult women. Therefore, it is unlikely that the observation of a comparable sex-ratio in pre- and postpubertal patients with sepsis is due to the presence of high levels of testosterone or the absence of female sex. This is further supported by the observation that in adults the male preponderance of severe sepsis is lower in the age group with highest testosterone levels, in adolescents, and adult patients below 30 years of age [12,16]. In contrast, a strong male preponderance is found in low birth-weight infants [12]. Neonates are not included in our study.

Since higher levels of male sex hormones are no explanation for the observed gender difference, it is likely that variations of X-chromosomal encoded regulatory factors of the immune system are contributing to a proinflammatory shift in immune response. These gender effects seemingly are not independent from the causative organism.

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SELECTIVE PULMONARY VASODILATION

PART A

Randomized controlled trial of aerosolized prostacyclin therapy in children with acute lung injury

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ABSTRACT

Objectives: To investigate whether aerosolized prostacyclin improves oxygenation in children with acute lung injury.

Design: Double-blind, randomized, and placebo-controlled trial.

Setting: Pediatric intensive care unit at a university hospital.

Patients: Fourteen children with acute lung injury defined by the criteria of an American-European Consensus Conference.

Interventions: Aerosolized prostacyclin (epoprostenol sodium) by stepwise increments of different doses (10, 20, 30, 40, and 50 ng/kg/min) vs. aerosolized normal saline (placebo).

Measurements and Main Results: Before the start of the study, and before and after each dose of prostacyclin/placebo, the following variables were measured: arterial blood gases, heart rate, mean arterial blood pressure, and ventilator settings required. Changes in oxygenation were measured by calculation of the oxygenation index (mean airway pressure $\times$ 100 $\times$ PaO₂/FIO₂). After treatment with aerosolized prostacyclin, there was a significant 26% (interquartile range, 3%, 35%) improvement in oxygenation index at 30 ng/kg/min compared with placebo ($p < 0.001$). The response to prostacyclin was not the same in all children. We saw an improvement of $>20\%$ in eight of 14 children (i.e., responders), and the number needed to treat was 1.8 (95% confidence interval, 1.2-3.2). No adverse effects were observed.

Conclusions: Aerosolized prostacyclin improves oxygenation in children with acute lung injury. Future trials should investigate whether this treatment will positively affect outcome.

INTRODUCTION

The main clinical problem in patients suffering from acute lung injury (ALI) and its most severe form, the acute respiratory distress syndrome (ARDS), is severe hypoxemia that is associated with a high mortality rate and long-term sequelae [1-5]. Mechanical ventilation is the only lifesaving supportive measure to ensure adequate ventilation and to treat hypoxemia. Although there have been advances in ventilatory strategies to reduce ventilation-induced lung injury [6], any therapeutic modality that secures and improves oxygenation may further contribute to reduce injurious ventilator settings or high (toxic) concentrations of inspired oxygen, even though this cannot imply long-term improvement. However, until now no additional therapy (e.g., pharmaceutical) has been established.

Theoretically, aerosolized prostacyclin has this potential. In uncontrolled case series of adult patients with ARDS, aerosolized prostacyclin improved oxygenation by selective pulmonary vasodilation [7-9]. It compensates for the loss of the vasodilatory properties in the pulmonary vascular endothelium and counteracts the thromboxane-mediated vasoconstriction and platelet aggregation [1,10-13], which are the basic mechanisms causing hypoxemia in patients with ALI. Aerosolized prostacyclin redistributes blood flow from poorly or nonventilated areas to well-ventilated areas, thereby decreasing veno-venous mismatching and intrapulmonary shunting. In patients with pulmonary hypertension, aerosolized prostacyclin decreases pulmonary artery pressures. Pharmacologically, it acts immediately by receptor-mediated increase of 3':5'-cyclic adenosine monophosphate leading to relaxation of smooth muscle cells in the vascular endothelium. It acts immediately and its half-life time is short (2 min) due to rapid metabolism in the liver [14]. Prostacyclin can be applied easily by means of a standard nebulizer system [8].

However, no placebo-controlled study has been performed to evaluate this therapy in children. Therefore, we investigated whether aerosolized prostacyclin would sufficiently improve oxygenation in children with ALI and compiled a dose-response protocol.

MATERIALS AND METHODS

The study was conducted between June 1, 1999, and September 1, 2002, following the approval of the institutional review board. Informed consent from the parents was obtained before enrolling their children in the study. Children admitted to our pediatric intensive care unit requiring mechanical ventilation were screened each day to establish whether they fulfilled the criteria for ALI as defined by the American-European consensus conference in 1994 [1]. These criteria included a) acute onset of respiratory failure; b) $\text{PaO}_2/\text{FIO}_2$ ratio ≤ 300 torr; c) no clinical signs of atrial hypertension; and d) bilateral infiltrates on chest radiographs. For the purpose of the present study, criteria 2 and 3 were further elaborated; that is, a) we included only those patients with a $\text{PaO}_2/\text{FIO}_2$ ratio ≤ 300 torr in two consecutive measurements with a minimum interval of 8 hrs; and b) because pulmonary artery occlusion pressure is not routinely measured in critically ill children, we suspected left atrial hypertension clinically [1]: a history compatible with the possibility of left ventricular failure and signs of central venous congestion (rapid increase in central venous pressure, hepatomegaly, ascites and peripheral edema, jugular venous congestion). In these cases or in cases where there was doubt about the cardiac function or when patients were receiving inotropic/vasopressor support, we performed echocardiography. We did not include children with a decreased shortening fraction $< 30\%$, mitral regurgitation, and/or enlarged left atrium, whom we suspected to have raised left atrial pressure and cardiogenic pulmonary edema [15,16]. Furthermore, children were included only after the first 24 hrs of admission, when they are prone to multiple interventions (e.g., aggressive fluid resuscitation, frequent changes in inotropic/vasopressor dosages, etc.). These interventions can disturb oxygen uptake and might falsely lead to the diagnosis of ALI. Furthermore, we classified ALI due to the localization of the trigger of lung injury in primary (intrapulmonary) and secondary (extrapulmonary) lung injury [17].

Children were excluded from the study if one or more of the following criteria were present: thrombocytopenia ($< 50,000/\mu\text{L}$), bleeding diathesis, activated partial thromboplastin time of > 43 secs, intracranial hemorrhage, congenital heart disease, acute renal failure, chronic lung disease or poor prognosis with the probability of

death, or withdrawal of therapy within the following 24 hrs. After inclusion, nebulization was started within the following 12 hrs to measure an early treatment effect.

Design

After enrolment into the study, in all children nebulization was started with normal saline during 20 min, and thereafter baseline variables were recorded: heart rate, mean arterial blood pressure, arterial blood gases, and ventilator settings required. The patients were then randomized by sequentially numbered envelopes, following a crossover randomization procedure described by Altman [18]. This resulted in two groups: one group (group 1) in which children were first treated with five doses of aerosolized prostacyclin followed by normal saline (designated as placebo) and a second group (group 2) in which the children were initially treated with five doses of normal saline followed by aerosolized prostacyclin. When the study was stopped, 14 patients were included.

Aerosol Preparation

We prepared prostacyclin (epoprostenol sodium, a synthetic analog of the natural prostacyclin; Flolan; Glaxo-SmithKline B.V., Zeist, The Netherlands) following the producer's recommendations (pH of 10.2) in concentrations that would allow us to test different doses by stepwise increments (i.e., 10, 20, 30, 40, and 50 ng/kg/min). Investigators and caregivers were blinded to the assignment of the patients. Our pharmaceutical laboratory prepared both treatment and placebo drugs in vials with the same external appearance. Before preparing the concentrations, we assessed the rate of aerosol delivery in milliliters per minute for each patient during the baseline nebulization with normal saline. Each dose was administered during a 20-min period, and then blood samples were drawn and the respiratory and circulatory variables mentioned previously were determined. To allow for washout, there was a 30-min period between the prostacyclin and the placebo nebulization and a 5-min period between each dose increment.

Aerosol Delivery

For the purpose of this study, we included only those children who were intubated with endotracheal tubes with an internal diameter > 3.5 mm, because the smaller the tube size the smaller the amount of medication deposited in the alveoli [19]. In addition, because alveolar aerosol deposition is optimal with a particle size of $2\text{--}5\text{ }\mu\text{m}$ mass mean aerodynamic diameter [20], we performed nebulization with an ultrasonic nebulizer (SUN 145, Siemens-Elema AB, Solna, Sweden, output range $0.1\text{--}0.5\text{ mL/min}$), which produces a particle size of approximately $4.0\text{ }\mu\text{m}$ mass mean aerodynamic diameter (manufacturer's data). The nebulizer is connected to the inspiratory limb of the ventilator system, and the medication is introduced through a syringe port without disconnection from the ventilator. With an ultrasonic system, no extra gas flow interferes with the preset ventilatory variables.

The effect of aerosol delivery in ventilated patients also depends on the amount of recruited lung volume. On our pediatric intensive care unit, children are ventilated with positive end-expiratory pressure titrated based on FIO_2 , and recruitment maneuvers are performed for collapsed lung regions.

Table 1. Underlying causes of acute lung injury and outcome in the study population

Patient	Diagnoses	Secondary lung injury ^a	Survival
1	Multiple trauma with lung contusion	No	Yes
2	Severe multiple-organ failure due to cytochrome C oxidase deficiency (complex-IV)		
	Cerebral coma	Yes	No
3	Severe multiple-organ failure due to respiratory chain defect with status epilepticus and coma	Yes	No
4	Meningococcal septic shock	Yes	Yes
5	Multiple trauma	Yes	Yes
6	Viral pneumonia (adenovirus)	No	Yes
7	Meningococcal septic shock	Yes	Yes
8	Severe multiple-organ failure due to metabolic disorder with hypoglycemia and status epilepticus	Yes	No
9	Sickle cell crisis with acute chest syndrome	No	Yes
10	Myasthenia gravis with bacterial pneumonia (Staphylococcus aureus)	No	Yes
11	Hepatic teratoma	Yes	Yes
12	Aspiration pneumonia	No	Yes
13	Streptococcal toxic shock syndrome	Yes	Yes
14	Lamp oil aspiration	No	Yes

^aIn secondary lung injury the trigger of lung injury is extra-pulmonary (e.g., sepsis) and in primary lung injury the trigger is intra-pulmonary (e.g., pneumonia) Reference (17).

Table 2. Patients' characteristics and respiratory/physiological parameters at study inclusion

Patients (n=14)	Values at inclusion ^a
Age, months	54.0 (13.3, 104.5)
Weight, kg	18.0 (10.0, 31.5)
Intensive Care Unit day of study inclusion	3.0 (1.0, 5.3)
Tube size, internal diameter, mm	5 (4, 6)
Positive inspiratory pressure, cm H ₂ O	26 (24, 30)
Mean airway pressure, cm H ₂ O	17 (15, 19)
Positive end-expiratory pressure, cm H ₂ O	10 (7, 10)
Tidal volume, mL/kg	8.5 (6.3, 9.8)
FIO ₂	0.50 (0.45, 0.55)
Oxygenation index	10.0 (7.8, 14.5)
PaO ₂ /FIO ₂ , torr	194 (120, 219)
SpO ₂ , %	96 (94, 97)
PaCO ₂ , torr	46 (39, 62)
Heart rate, beats per minute	140 (103, 105)
Mean arterial blood pressure, mm Hg	75 (61, 83)
Arterial pH	7.39 (7.32, 7.46)

SpO₂, percutaneous oxygen saturation; paCO₂, partial arterial pressure of carbon dioxide;
 Oxygenation index, mean airway pressure X fraction of inspired oxygen X 100/ PaO₂

^a Median (interquartile range). To convert torr to kPa, multiply the value by 0.1333.

Table 3. Individual values of the oxygenation index (OI) at different doses prostacyclin compared with the mean values of the OI for all five doses normal saline

Patient number	Mean value of all five doses normal saline	Prostacyclin doses, ng/kg/min				
		10	20	30	40	50
Group I						
2	7.2	5.5	6.0	6.3	6.6	7.0
3	6.9	9.2	9.2	7.2	6.8	6.4
4	8.4	9.5	10.0	10.3	8.9	8.6
5	1.7	2.0	2.3	1.8	1.6	1.6
6	9.8	5.6	4.8	6.2	5.8	7.0
9	6.7	6.6	7.7	6.4	6.8	6.3
10	10.3	9.7	8.6	7.7	8.3	7.5
13	12.2	12.4	10.5	9.7	9.4	10.0
Group II						
1	18.9	11.0	9.5	9.0	9.5	9.0
7	24.0	18.6	19.3	20.2	19.6	20.5
8	21.3	19.4	15.7	13.8	17.7	17.2
11	19.6	17.7	15.4	12.6	15.3	15.3
12	13.2	14.7	14.7	10.6	9.9	12.1
14	7.6	6.9	6.7	5.7	10.9	9.9

Group I, prostacyclin followed by normal saline; group II, normal saline followed by prostacyclin.

Table 4. Oxygenation index, dose-dependent differences (prostacyclin vs. normal saline) per randomization group

Dose prostacyclin ng/kg/min	Group I (n=8)		Group II (n=6)		Mann-Whitney U test Z-value	p-value
	Prostacyclin followed by		Normal saline followed by			
	normal saline median (IQR)	prostacyclin median (IQR)	prostacyclin median (IQR)	normal saline median (IQR)		
10	-0.1 (-1.2; 1.9)		-1.3 (-3.3; -0.2)		-1.228	.114
20	0.3 (-1.1; 1.7)		-4.8 (-9.3; -0.5)		-2.066	.021
30	0.4 (-0.2; 2.7)		-6.0 (-7.6; -3.9)		-3.037	.001 ^a
40	0.4 (0; 1.9)		-3.9 (-5.0; -2.6)		-2.197	.014
50	0.6 (0.3; 1.6)		-2.5 (-4.9; -0.2)		-2.066	.021

IQR, interquartile range; positive values in group I and negative values in group II indicate improvement in oxygenation index.

^a p<.01 (one-tailed) was considered significant.

Table 5. Physiological parameters and ventilator settings during nebulization with prostacyclin vs. normal saline

	Group I (n=8)		Group II (n=6)		Mann-Whitney U test	
	Prostacyclin followed by normal saline median (IQR)		Normal saline followed by prostacyclin median (IQR)		Z-value	p-value
Heart rate, bpm	3.3 (-2.2, 7.0)		-6 (-4.2, 0.8)		-1.229	.228
MAP, mm Hg	-3.0 (-8.2, 3.2)		-1.9 (-6.0, -0.2)		-.143	.945
FIO ₂	0 (0, 0.02)		-0.05 (-0.2, 0.01)		-.958	.354
Mean airway pressure, cm H ₂ O	-0.2 (-1.0, 0.6)		0 (0, 0.02)		-.924	.414
Arterial pH	-0.044 (-0.044, 0.027)		0.033 (0.016, 0.042)		-1.422	.181

IQR, interquartile range; MAP, mean arterial blood pressure; bpm, beats per minute.

^a p<.05 (two-tailed) was considered significant.

Outcome Variables

We calculated the oxygenation index (OI, mean airway pressure X 100 X PaO₂ /FIO₂) to document changes in oxygenation. The OI has been shown to be a reliable measure to assess the severity of hypoxemic respiratory failure in mechanically ventilated children, because it takes into consideration changes in mean airway pressure [1,21]. Therefore, the attending physician was allowed to adjust the ventilator settings during the study period for each patient as required. To document adverse effects on hemodynamic variables (spillover of the aerosol), we analyzed differences in heart rate and mean arterial blood pressure between the placebo and prostacyclin period. To limit the influence of confounding factors, we monitored the changes of the following variables during the study period: arterial blood pH (possible absorption of the alkaline buffer), FIO₂, tidal volume, peak inspiratory pressure, positive end-expiratory pressure, mean airway pressure, and partial arterial pressure of carbon monoxide. These variables were measured at baseline and before and after the administration of each dose of placebo or prostacyclin.

During the study period (after June 1, 1999), the technology of new ventilators (EVITA 4 Fa. Draeger, Lübeck, Germany) allowed us to observe in nine of the 14 patients flow/volume (F/V) curves on the ventilator screen. Before and after nebulization of each dose, the presence of flow limitation in the expiratory limb of the F/V curve was interpreted as airway hyperreactivity due to aerosol treatment.

Statistical Analysis

Based on the crossover design, all 14 patients had to be analyzed within their randomization groups. In group 1, OI values for each dose prostacyclin were subtracted from the OI values for the same dose of placebo (positive differences indicate improvement of OI), and in group 2, OI values for each dose of placebo were subtracted from the OI values for the same dose of prostacyclin (negative differences indicate improvement of OI). To test the effectiveness of prostacyclin aerosol for each dose, the differences between prostacyclin and placebo in group 1 were compared with the differences between placebo and prostacyclin in group 2 applying the Mann Whitney U-test. We hypothesized that prostacyclin aerosol would

improve the OI. To account for the number of multiple comparisons (five dosages), $p < 0.01$ was considered significant.

For clinical use, the significant improvements of the OI were also expressed as percentage of improvement. In terms of responders and nonresponders, we calculated the number needed to treat to observe at least a 20% improvement of the OI in a single patient.

To test for changes in hemodynamic variables and in ventilator settings during the study, the same statistical procedure as used for the OI was followed for heart rate, mean arterial blood pressure, FIO_2 , mean airway pressure, and pH ($p < 0.05$, two-tailed). Data were analyzed using the SPSS software for Windows, version 10.0 (SPSS, Chicago, IL).

RESULTS

Fifteen children met the inclusion criteria. One parent refused participation of her daughter in the study. Of the remaining 14 children (ten boys and four girls), eight received prostacyclin before normal saline (group 1) and six received normal saline before prostacyclin (group 2). Tables 1 and 2 give data on the underlying diseases, distribution of primary (six of 14) and secondary (eight of 14) lung injury, outcomes, and the patients' characteristics at study inclusion. Table 3 gives the individual OI values for each dose of prostacyclin. We found a significant improvement of the OI at 30 ng/kg/min ($p < .001$, Table 4). The median extent of this improvement was 26% (interquartile range, 3%, 35%; or median, -2.5; interquartile range, -5.8, -0.2). A trend toward significance was observed at the 20, 40, and 50 ng/kg/min doses (Table 4). Furthermore, analysis at 30 ng/kg/min aerosolized prostacyclin revealed eight responders at a cut-off of 20% improvement (number needed to treat, 1.8; 95% confidence interval, 1.2-3.2). There was no difference in treatment effect between children with primary or secondary lung injury ($p = .66$). During the study period, no changes in F/V curves, heart rate, mean arterial blood pressure, ventilation, and respiratory variables were observed (Table 5).

DISCUSSION

The primary aim of the present study was to investigate whether aerosolized prostacyclin might improve oxygenation in children with ALI. Based on the statistically significant 26% improvement, we believe that this effect is clinically relevant and that it confirms the principle of selective pulmonary vasodilation in this category of patients.

A decade ago, Walmrath et al. [8] were the first to show in three adult patients with acute respiratory distress syndrome that aerosolized prostacyclin decreases intrapulmonary shunts and increases oxygenation ($\text{PaO}_2/\text{FIO}_2$ ratio) by about 40%. Later clinical studies confirmed these findings [10,22-24]. Until now, only one case report has presented data on three children with acute respiratory distress syndrome: in that study, aerosolized prostacyclin improved oxygenation by 18-71% (dose range, 2-20 ng/kg/min) [25].

The fact that we found a significant improvement only at 30 ng/kg/min and values close to significance at the 20, 40, and 50 ng/kg/min doses (Table 4) might be because 14 patients are still too small a number to demonstrate significant responses at more doses. With respect to the “starting dose,” dose responses to aerosols are also dependent on the size of the endotracheal tube used, and the smaller the tube the smaller the amount of aerosol deposition [19,20]. In our pediatric study population, smaller tube sizes (median internal diameter, 5 mm) have been used compared with those used in adult patients (internal diameter, 7-9.5 mm). This might explain why, in an adult study, dose of 30 ng/kg/min already showed significant improvements [7]. Differences in response are also reported for different patterns of ALI: primary vs. secondary lung injury [26], with the largest response in secondary lung injury, possibly due to less consolidated lung regions. However, our results do not confirm this latter observation.

Adverse Effect

Due to reports about hemodynamic adverse effects with doses >100 ng/kg/min (spillover to systemic circulation), we limited the maximum dose to 50 ng/kg/min [8,22,27]. Because no changes in hemodynamics occurred (Table 5), a relevant

spillover can be excluded. Although prostacyclin is a very strong inhibitor of platelet aggregation [27,28], no bleeding incident was observed in any of our studied patients. Despite the alkaline pH of the prostacyclin solution (pH 10.2), other studies did not report adverse effects on the airways [29-31] and in nine of 14 children of the present study the F/V curves did not indicate airway hyperreactivity. In one other study, the mild tracheitis reported in two dogs needs to be confirmed by additional investigations [32].

Inhaled Nitric Oxide

One study in children compared inhaled nitric oxide with aerosolized prostacyclin and found a similar short-term improvement of oxygenation for both substances [25]. However, other investigators did not find convincing evidence that long-term treatment affects outcome, and concerns remain about its toxicity [33-40]. Moreover, compared with inhaled nitric oxide, aerosolized prostacyclin has no proven adverse effects [29-31], is cheaper, and is easier to administer. The estimated costs per hour for inhaled nitric oxide are \$125 [41] and for aerosolized prostacyclin are \$13 in a child weighing, for example, 18 kg at a dose of 30 ng/kg/min (manufacturer's data). However, these costs are based on the current approved indications for prostacyclin and will need to be recalculated after national registration for this indication.

The limitations of our study are threefold. First, with a larger number of patients, significant improvements of the OI might have been observed with more doses, and patient subgroups with different response might have been identified. Second, the effects of aerosolized prostacyclin on airway reactivity were evaluated by inspection of F/V curves in nine of 14 patients and were not measured in absolute numbers of airway resistance by standardized pulmonary function testing. Third, the study was designed to measure a surrogate (i.e., oxygenation) and no other clinically relevant end points, such as duration of ventilation, time of hospitalization, and costs. Future trials should investigate whether improved oxygenation due to aerosolized prostacyclin will positively affect clinically relevant end points, such as high concentrations of FIO₂ (e.g., oxygen toxicity) [42,43], the number of days on ventilation and

mechanical ventilation-induced lung injury, the use of inhaled nitric oxide therapy (e.g., toxic effects) [33-35], and pediatric intensive care unit costs [41]. In this context, newer drugs (e.g., endothelin-1 receptor antagonist or ilomedin), which have recently been shown to reduce pulmonary hypertension in adult patients, will also be tested [44-46]. Whether these drugs are as effective as aerosolized prostacyclin for selective pulmonary vasodilation needs to be proven against the advantages of aerosolized prostacyclin for the critically ill patient (i.e., short half-time, very rapid titration upward or downward, anti-inflammation, and safety). Due to the complexity of ALI, we believe that not every single therapy will positively affect long-term outcome. Therefore, future studies should also be designed to address the additive effects of different new approaches (e.g., surfactant, steroids, new ventilation strategies, and therapy with an SVP) on overall and long-term outcome of these patients.

CONCLUSIONS

Aerosolized prostacyclin improves oxygenation in children with ALI. Future trials are needed to investigate whether this therapy will improve outcome of these young patients.

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SELECTIVE PULMONARY VASODILATION

Part B

Combination of inhaled nitric oxide and intravenous prostacyclin for successful treatment of severe pulmonary hypertension in a patient with ARDS

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Sir: I would like to comment on this case report on the treatment of pulmonary hypertension (PH) with a selective [nitric oxide (iNO)] and a non-selective [intravenous prostacyclin (i.v. PGI₂)] pulmonary vasodilator [1]. The authors showed in Table 1 that this therapy decreased the pulmonary artery pressure (PAP) in a patient with the adult respiratory distress syndrome (ARDS), but i.v. PGI₂ also caused deterioration in oxygenation, compared with the iNO therapy alone. Because the arterial oxygen tension (PaO₂) remained above the pre-treatment level (=pre-iNO therapy) the authors concluded that their combination therapy improved gas exchange and, therefore, should be recommended. I doubt whether this conclusion should be accepted because of the negative effect of i. v. PGI₂ on oxygenation in contrast to the small beneficial effect on PAP.

Basically, i.v. PGI₂ dilates not only the ventilated pulmonary blood vessels but also the non-ventilated areas, augmenting intrapulmonary shunt volume [2]. In this way, not only will PAP be decreased but oxygenation will deteriorate as well. Therefore, research now focuses on therapy with selective intrapulmonary vasodilators, i.e. iNO and PGI₂ aerosol (own study in progress). This selective therapy of PH lowers PAP but not PaO₂. In order to optimize this treatment, I would like to add some suggestions.

When a ventilated patient does not respond any longer to iNO (gold standard in testing PH), one should ask oneself whether PH is still reversible and accessible to other vasodilators. If not, other therapies should be evaluated. In general, we still do not know why some patients do not respond to iNO. At least, in ventilated patients we know that some factors influence the effect of inhalation drugs, including iNO and PGI₂ aerosol. Firstly, the appropriate dosage of iNO is still under discussion. Therefore, increasing iNO on a trial basis is recommended [3]. Secondly, the inhaled drug must always get into the lungs. This can only be achieved by means of proper ventilator strategy recruiting the lungs and preventing atelectasis. And last, other complications (i. e. pulmonary embolism) which can increase PAP, imitating ineffective iNO therapy, must be excluded.

Combining two vasodilators that function by different mechanisms is a smart and worthwhile idea. However, I would not recommend the combination with a nons-

elective pulmonary vasodilator, but optimize the selective approach, where PGI_2 aerosol is a promising new therapy to reach the final goal: better overall organ oxygenation and function in patients with ARDS.

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ALVEOLAR FIBRINOLYSIS AND MECHANICAL VENTILATION

Part A

Alveolar fibrinolytic capacity suppressed by injurious mechanical ventilation

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ABSTRACT

Objective: To investigate the effect of mechanical ventilation on alveolar fibrinolytic capacity.

Design and setting: Randomized controlled animal study in 66 Sprague-Dawley rats.

Subjects and interventions: Test animals received intratracheal fibrinogen and thrombin instillations; six were killed immediately (fibrin controls), and the others were allocated to three ventilation groups (ventilation period: 225 min) differing in positive inspiratory pressure and positive end-expiratory pressure, respectively: group 1, 16 cmH₂O and 5 cmH₂O (n=17); group 2, 26 cmH₂O and 5 cmH₂O (n=16); group 3, 35 cmH₂O and of 5 cmH₂O (n=17). Ten animals that had not been ventilated served as healthy controls.

Measurements and results: After animals were killed, we measured D-dimers, plasminogen activator inhibitor (PAI) 1, and tumor necrosis factor α in the bronchoalveolar lavage fluid and calculated lung weight and pressure/volume (P/V) plots. The median D-dimer concentration (mg/l) decreased with increasing pressure amplitude (192 in group 1, IQR 119; 66 in group 2, IQR 107; 29 in group 3, IQR 30) while median PAI-1 (U/ml) increased (undetectable in group 1; 0.55 in group 2, IQR 4.55; 3.05 in group 3, IQR 4.85). PAI-1 level was correlated with increased lung weight per bodyweight (Spearman's rank correlation 0.708). Tumor necrosis factor α concentration was not correlated with PAI-1 level.

Conclusions: Alveolar fibrinolytic capacity is suppressed during mechanical ventilation with high pressure amplitudes due to local production of PAI-1.

INTRODUCTION

intraalveolar fibrin-rich hyaline membranes are the distinguishing microscopic feature of numerous acute diffuse lung diseases including acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [1,2]. In ALI acute insults (e.g., sepsis and pneumonia) trigger an inflammatory response causing increased capilloalveolar permeability for, e.g. fibrinogen. Alveolar cells (i.e., macrophages and epithelial cells) are stimulated directly by bacterial endotoxins or by proinflammatory mediators, with a central role played by tumor necrosis factor (TNF) α in producing procoagulant (i.e., factor VII and tissue factor) and antifibrinolytic proteins resulting in alveolar fibrin depositions. The degree and persistence of alveolar fibrin depends predominantly on local production of plasminogen activator inhibitor (PAI)-1, the direct antagonist of the fibrinolytic system [3,4,5,6,7,8].

Alveolar fibrin membranes inactivate and incorporate surfactant leading to disturbance of gas exchange, impairment of lung mechanics, and aggravation of capilloalveolar leakage [9]. Furthermore, they may contribute to the inflammatory response observed in ALI and also initiate a fibroproliferate state with *de novo* inflammation, fibrosis, and long-term compromised pulmonary function in infants, children, and adults after acute respiratory failure [10]. Under normal circumstances fibrin membranes are resolved within minutes by plasmin [11,12]. However, during ALI additional insults (e.g., hemorrhagic shock, infections) may prolong disturbed alveolar fibrin turnover [13]. In patients with ALI mechanical ventilation, although mandatory, is considered a secondary insult for lung damage. Mechanical ventilation with high tidal volume and/or high pressure amplitude without sufficient positive end-expiratory pressures (PEEP) causes shear stress on lung tissue which stimulates *de novo* production of proinflammatory cytokines in, for example, alveolar cells [14,15,16]. This so-called mechanotransduction-related lung inflammation is believed to be the major determinant of ventilation-induced lung injury (VILI) and its associated morbidity and mortality [17,18,19,20,21]. Despite this understanding, there is still a tremendous lack of knowledge about the mechanisms involved [22]. For example, it has not yet been investigated whether mechanical ventilation interferes with alveolar fibrin turnover or with the natural capacity of alveolar fibrin resolution [23,24].

Therefore we investigated the effects of mechanical ventilation with various high pressure amplitudes on alveolar fibrinolytic capacity in a rat model of iatrogenic intraalveolar fibrin formation.

MATERIALS AND METHODS

The study was approved by the Animal Committee of the Erasmus University Rotterdam. Care and handling of the animals were in accordance with European Union guidelines. The experiments were performed at the Department of Anesthesiology, Erasmus Medical Centre-Faculty in male Sprague-Dawley rats (n=66) with a bodyweight (BW) of 287 ± 3 g (IFFA Credo, The Netherlands).

Experimental protocol

Rats were anesthetized with nitrous oxide, oxygen and isoflurane (65/33/2%), tracheotomized, and had a catheter inserted into a carotid artery. Anesthesia was maintained with hourly intraperitoneal injections of pentobarbital sodium (60 mg/kg; Nembutal, Algin, Maassluis, The Netherlands). Muscles were relaxed with 2 mg/kg pancuronium bromide (Pavulon; Organon Technika, Boxtel, The Netherlands) intramuscularly hourly. After muscle relaxation all animals were connected to a ventilator (Servo Ventilator 300; Siemens-Elema, Solna, Sweden) in a pressure-controlled mode with positive inspiratory pressure (PIP) of 12 cmH₂O and PEEP of 2 cmH₂O, frequency of 30 breaths/min, inspiratory to expiratory ratio of 1:2, and fractional inspired oxygen tension of 1.0. Body temperature was kept within normal range by means of a heating pad.

After 15-min stabilization intraalveolar fibrin formation was generated by intratracheal instillation of human fibrinogen (40 mg/kg, Sanquin, Amsterdam, The Netherlands) followed 1 min thereafter by human thrombin (10 µg/kg, a generous gift from Dr. W. Kisiel, University of New Mexico, Albuquerque, N.M., USA). Both fibrin and thrombin were diluted to a maximum volume of 1 ml (with normal saline). Doses of fibrinogen and thrombin were derived from the literature [25] and our own pilot experiments. For optimal fibrin deposition PIP was increased to 26 cmH₂O

just before, and intratracheal air (10 ml/kg BW) was instilled three times just after instillation of fibrin. After instillation PIP was adjusted immediately according to the allocation of the animals to the different treatment groups. Arterial blood gases were taken from a carotid artery; just before fibrinogen/thrombin instillation and additional samples were taken at 15 min and every 30 min thereafter. Blood gases were measured using conventional methods (ABL555, Radiometer, Copenhagen, Denmark). Blood pressure was monitored through the carotid artery before instillation and at 15 and every 30 min thereafter for 225 min after fibrin instillation. Before measurements the rats were killed after 225 min of mechanical ventilation with an intra-arterial overdose of pentobarbital sodium (600 mg/kg BW pentobarbital sodium).

Study groups

Ten rats were killed immediately after the surgical procedure and served as healthy control rats. Animals which received instillations of fibrinogen/thrombin were randomly allocated to four groups. In the group designated as “fibrin controls” six animals were killed 5 min after the instillation procedure; these served as controls for the ventilation groups with respect to the D-dimer and PAI-1 measurements. In the three other groups animals were ventilated with various high pressure amplitudes: group 1, PIP 16 cmH₂O and PEEP 5 cmH₂O (n=17); group 2, PIP 26 cmH₂O and PEEP 5 cmH₂O (n=16); and group 3, PIP 35 cmH₂O and PEEP 5 cmH₂O (n=17). The ranges of tidal volumes (ml/kg) for each group were: group 1 13–16, group 2 22–26, and group 3 28–32.

Bronchoalveolar lavage and lung mechanics

The time at which bronchoalveolar lavage was performed was defined at the moment when rats died prior to or at the end of the study period at 225 min. After the animals were killed, the thorax and diaphragm were opened (to eliminate the effect of chest wall compliance and intra-abdominal pressure) and a static pressure-volume (P/V) plot from the lung was calculated using conventional techniques [26]. Maximal compliance (C_{max}) was defined as the steepest part of the P/V deflation

curve and was determined separately for each animal. Measurements were not completed in 14 rats due to air leakage during the test. After the P/V plot bronchoalveolar lavage was performed with normal saline (30 ml/kg heated to 37°C) and reaspirated three times, and the recovered supernatant fluid was stored at -80°C until further processing. No bronchoalveolar lavage was performed in the animals in which histology was performed (one per group).

Fibrinolysis measurements

D-dimer levels in the bronchoalveolar lavage fluid (BALF) were quantitated by a sandwich-type enzyme-linked immunosorbent assay (Asserachrom D-dimer, Diagnostica Stago, Asnières-sur-Seine, France). PAI-1 activity in BALF was determined on an automated coagulation analyzer (Behring Coagulation System, Marburg, Germany) with reagents and protocols from the manufacturer. This assay determines the urokinase-inhibiting activity of the sample.

Table 1. Physiological parameters during study period at different time points

	Survivors	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	pH	MAP (mmHg)
Before fibrin instillation	50				
Group I	17	485 ± 11	36 ± 3	7.42 ± 0.02	130 ± 9
Group II	16	540 ± 12	31 ± 2	7.52 ± 0.02 [†]	140 ± 5
Group III	17	512 ± 24	31 ± 2	7.51 ± 0.02 [†]	131 ± 6
15 minutes	49				
Group I	17	336 ± 30	50 ± 3	7.28 ± 0.02	119 ± 7
Group II	16	412 ± 20	33 ± 2	7.46 ± 0.02 [†]	125 ± 3
Group III	16	408 ± 24	39 ± 12	7.52 ± 0.02 [†]	119 ± 5
45 minutes	49				
Group I	17	353 ± 32	48 ± 4	7.30 ± 0.02	111 ± 10
Group II	16	311 ± 33	34 ± 1 [†]	7.46 ± 0.01 [†]	120 ± 5
Group III	16	234 ± 29 [†]	28 ± 2 [†]	7.50 ± 0.02 [†]	96 ± 4 [‡]
75 minutes	49				
Group I	17	364 ± 32	39 ± 3	7.37 ± 0.03	101 ± 10
Group II	16	304 ± 38	37 ± 2	7.44 ± 0.02	105 ± 8
Group III	16	105 ± 20 [†]	32 ± 2	7.40 ± 0.02	71 ± 5 ^{†,‡}
105 minutes	44				
Group I	15	399 ± 33	37 ± 3	7.41 ± 0.03	108 ± 10
Group II	16	263 ± 36 [†]	38 ± 3	7.39 ± 0.03	92 ± 8 [‡]
Group III	13	78 ± 9 ^{†,‡}	34 ± 2	7.34 ± 0.02	62 ± 7 [†]
135 minutes	37				
Group I	15	426 ± 37	38 ± 3	7.41 ± 0.03	89 ± 11
Group II	14	289 ± 36 [†]	38 ± 2	7.39 ± 0.02	98 ± 5
Group III	8	69 ± 8 ^{†,‡}	37 ± 3	7.33 ± 0.03	54 ± 4 ^{†,‡}
165 minutes	34				
Group I	14	412 ± 37	38 ± 3	7.42 ± 0.03	98 ± 12
Group II	14	349 ± 100	40 ± 3	7.34 ± 0.03	85 ± 5
Group III	6	68 ± 10 [†]	34 ± 2	7.44 ± 0.05 [†]	57 ± 8 [†]
195 minutes	26				
Group I	9	457 ± 20	39 ± 3	7.41 ± 0.02	110 ± 9
Group II	14	225 ± 40 [†]	42 ± 5	7.32 ± 0.06	69 ± 7 [†]
Group III	3	91 ± 12 [†]	36 ± 3	7.34 ± 0.06	60 ± 5 [†]
225 minutes	8				
Group I	3	450 ± 28	47 ± 6	7.40 ± 0.05	84 ± 9
Group II	4	198 ± 68	42 ± 7	7.32 ± 0.03	67 ± 10
Group III**	1	86	42	7.26	65

Group I: PIP 16 cmH₂O/PEEP 5 cmH₂O; Group II: PIP 26 cmH₂O/PEEP 5 cmH₂O;

Group III: PIP 35 cmH₂O/PEEP 5 cmH₂O; MAP, mean arterial pressure. Data are mean ± SE.

** Post hoc tests are not performed at time 225 minutes because at least one group has fewer than two cases.

† $p < 0.05$ vs. group I

‡ $p < 0.05$ vs. group II

Table 2 Characteristics of the study groups

	Lung weight (g)/kg BW	Survival time (min)
Controls (n=10)	6.0±0.4 (n=4)	--
Fibrin controls (n=6)	12.5± 0.7 (n=6)	--
Group I (n=17)	5.8±0.3 (n=12) [§]	172±11
Group II (n=16)	13.2±1.4 ^{§§} (n=14)	186±12 [†]
Group III (n=17)	16.2±1.6 ^{§§} (n=14)	122±13 [‡]

Group I, PIP 16 /PEEP 5 cmH₂O; group II, PIP 26/PEEP 5 cmH₂O; group III, PIP 35 /PEEP 5 cmH₂O.

Survival time, maximum survival time to kill at 225 minutes. Data are mean ± SE.

[§] $p < 0.05$ vs. all other groups except group II

^{§§} $p < 0.05$ vs. all other groups

[†] $p < 0.05$ vs. group III and vs. controls

[‡] $p < 0.05$ vs. groups I,II and vs. controls

The remaining urokinase is then assayed by activating plasminogen to plasmin and subsequent determination of plasmin's chromogenic activity. The assay is independent of variable concentrations of plasminogen, α_2 -antiplasmin, and fibrinogen in the sample. We cannot exclude that other urokinase inhibitors were determined; however, PAI-1 is the major inhibitor of urokinase. Furthermore, the results of this assay are correlated very well with those of methods based on the inhibition of urokinase-type plasminogen activator. The concentration of urokinase-type plasminogen activator in the BALF was not assessed, but with these high concentrations of PAI-1 these are most likely extremely low.

TNF- α measurements

Levels of TNF- α in BALF were measured by a commercial enzymelinked immunosorbent assay (Rat TNF- α /TNFSF2, catalogue no. DY510, R&D Systems, Abingdon, UK).

Lung histology

To determine alveolar fibrin depositions samples were taken from all lung lobes and 30 fields were analyzed. The analyzing pathologist was not informed about the study purpose and was asked only to prepare rat lungs for illustration of alveolar fibrin deposition. For fixation 4% paraformaldehyde was instilled in the lungs under a positive airway pressure for lung recruitment of 10 cmH₂O. The specimens were embedded in paraffin, sectioned in tissue blocks from all lobes, and stained with hematoxylin and eosin.

For fibrin staining slides were deparaffinized, and endogenous peroxidase activity was quenched by a solution of methanol/0.03% H₂O₂ (Merck, Darmstadt, Germany). After digestion with a solution of pepsine 0.25% (Sigma, St. Louis, Mo., USA) in 0.01 M HCl, the sections were incubated in 10% normal goat serum (Dako, Glostrup, Denmark) and then exposed to biotin-labelled goat antihuman fibrinogen antibody (Ixell, Accurate Chemical & Scientific, Westbury, N.Y., USA). After washes slides were incubated in a streptavidin-ABC solution (Dako) and developed using 1% H₂O₂ and 3.30-diaminobenzidinetetra-hydrochloride (Sigma) in Tris-HCl. The sections were mounted in glycerin gelatin and counterstained with hematoxylin.

Statistics

Statistical analysis was performed using SPSS version 11.5 (SPSS, Chicago, Ill., USA). Data of physiological parameters are presented as mean $\pm$ standard error. Intergroup differences were analyzed by analysis of variance and Bonferroni's post-hoc test; differences at the level of $p < 0.05$ (one-tailed) were considered significant. For multiple comparisons Bonferroni's correction was used: $1-\alpha = 0.05/3 = 0.017$ and $p < 0.017$ (one-tailed) was considered significant. Data on D-dimer and PAI-1 are presented as median and interquartile range (IQR), and on the Kruskal Wallis test corrected for multiple comparisons differences at the level of $p < 0.017$ (one-tailed) were considered significant. The Mann Whitney U-test for comparisons between two groups was performed, and the level of $p < 0.05$ (one-tailed) was considered significant. In each ventilation group we calculated post hoc the correlations between D-dimer and PAI-1 concentrations in BALF with survival time. Linear regression analysis was performed to evaluate the relationship between ventilation pressure, D-dimer, PAI-1, and lung weight (g)/kg bodyweight (BW), with $p < 0.05$ being considered significant.

RESULTS

Lung injury

Table 1 shows the physiological parameters and their differences between the ventilation groups. To document the traumatic effects of mechanical ventilation on lungs we compared changes in lung weight and P/V curves of the ventilation groups with those of healthy controls (unventilated animals). We found that the mean lung weights per BW in groups 2 and 3 were significantly higher than those in healthy controls and group 1 (Table 2). In groups 2 and 3 lung function was impaired, as demonstrated by significantly lower values for the P/V plots (Figure 1); however, no differences were found between group 1 and healthy controls. The higher relative lung weight in "fibrin controls" was due to the fact that they were killed 5 min after the instillation procedure (2 ml volume load).

Fibrinolytic capacity

As shown in figure 2, fibrin-rich material was deposited in alveoli in fibrin-treated animals. Levels of D-dimers were below detection limit in healthy control rats (no instillation of fibrin, no mechanical ventilation). “Fibrin controls” (animals killed 5 min after the instillation procedure) showed a small but significant increase in D-dimer in BALF compared to healthy control rats: 0 vs. 3.4 mg/l (IQR 3.2). In animals that had been mechanically ventilated the median BALF level of D-dimer (median 192 mg/l; IQR 119) was highest in group 1 (lowest pressure amplitude). A further increase in pressure amplitude (groups 2 and 3) resulted in significantly lower D-dimer concentrations in BALF (Figure 3A, group 2 66 mg/l, IQR 107; group 3 29 mg/l, IQR 30); $p < 0.001$, Kruskal Wallis). BALF concentrations of PAI-1 were below detection limit in all control rats (with and without fibrinogen/thrombin instillation) and in rats ventilated with the lowest pressure amplitude (group 1; Figure 3B). In inverse relationship to the decrease in D-dimer levels, median BALF concentrations of PAI-1 increased the higher pressure amplitude during mechanical ventilation (group 2, 0.55 U/ml, IQR 4.55, $p = 0.007$; group 3, 3.05 U/ml, IQR 4.85, $p = 0.001$, Figure 3B).

Analysis of whether the measurement of fibrinolytic parameters depended on the duration of mechanical ventilation (time dependence) is shown in figure 4. In a post-hoc analysis no intragroup differences were found in D-dimer or PAI-1 regarding the time at which BAL measurements were performed ($p > 0.05$, analysis of variance). Linear regression revealed that PAI-1 levels were correlated with the increase in lung weight per BW ($R^2 = 0.496$; Spearman’s rank correlation coefficient 0.708, $p < 0.001$; figure 5).

Levels of TNF- α

TNF- α was undetectable in all controls, as in the majority of ventilated rats (12/16 in group 1, 4/15 in group 2, 10/16 in group 3). Group means did not differ significantly (0 pg/ml in group 1, IQR 1.5; 36 pg/ml in group 2, IQR 212; 0 pg/ml in group 3, IQR 58).

Figure 1. Effect of mechanical ventilation on lung function: P/V plots, lines connect group means $\pm$ SE at each step of 5 cmH₂O increase in airway pressure. X Healthy controls without fibrin instillation; enclosed X healthy controls with fibrin instillation; squares group 1 (16/5 cm H₂O); circles group 2 (26/5 cm H₂O); diamonds group 3 (35/5 cm H₂O). A Healthy controls without vs. controls with fibrin instillation; mean difference 117 $\pm$ 41 ml/kg. B Healthy controls vs. group 1; mean difference 66 $\pm$ 33 ml/kg ($p=0.21$). C Healthy controls vs. group 2; mean difference 170 $\pm$ 34 ml/kg ($p<0.001$). D Healthy controls vs. group 3; mean difference 241 $\pm$ 33 ml/kg ($p<0.001$). Mean difference $\pm$ SE between group 2 (B) and 3 (C) 70 $\pm$ 14 ml/kg ($p=0.023$) and that between controls with fibrin instillation and group 3 124 $\pm$ 33 ml/kg ($p=0.005$). * $p<0.05$ (analysis of variance)

Figure 2. Microscopy of representative fibrin-stained paraffin sections of rat lung tissue. **A** Healthy animals, normal lung tissue, no fibrin in the alveolar space. **B** Group 1 (16/5 cmH₂O) after 225 min of ventilation. **C** Group 2 (26/5 cmH₂O) after 195 min of ventilation. **D** Group 3 (35/5 cmH₂O) after 135 min of ventilation. Arrows intraalveolar fibrin depositions; original magnification x 140

Figure 3. Effect of mechanical ventilation on D-dimer and PAI-1 concentrations in BALF. Values are median, boxes represent interquartile range (25th–75th percentiles), * $p < 0.05$ was considered significant (Mann Whitney U-test). *N* indicates the number of animals. A: D-dimer, * $p < 0.05$ group 1, II and III vs. both control groups. B: PAI-1, * $p < 0.05$ group 2 and III vs. group 1 and both control groups.

Figure 4. Median D-dimer (A) and PAI-1 (B) levels at time of death (survival time, min) in BALF are shown for the three ventilation groups. Boxes interquartile range (25th–75th percentiles); *N*, number of animals used for analysis at each time point. There were no significant differences at the different time points between ventilation groups ($p > 0.05$, analysis of variance).

Figure 5. Linear regression: correlation between lung weight/body weight (BW) and PAI-1 concentrations in BALF ($p < 0.001$, $R^2 = 0.496$, Spearman's rank correlation coefficient 0.708).

DISCUSSION

The present study analyzed the effect of mechanical ventilation on the alveolar fibrinolytic capacity in an *in vivo* model of iatrogenic intraalveolar fibrin formation. Interestingly, mechanical ventilation with a small pressure amplitude (16/5 cmH₂O, group 1) neither altered lung weight nor caused production of PAI-1. These animals showed the highest D-dimer concentrations in BALF. In contrast, mechanical ventilation with larger pressure amplitudes (groups 2 and 3) resulted in significantly greater lung weight and depressed intraalveolar fibrinolysis. Since high levels of PAI-1 were directly related to the severity of lung injury (i.e., lung weight per BW), PAI-1 seems to be responsible for this observation.

Experimental model

Our experimental model to create intraalveolar fibrin formation is similar in some aspects to a model described previously [25]; doses were derived from the latter study and from pilot experiments in our laboratory. The illustrations from

microscopy (Figure 2) and the large amounts of D-dimers in BALF (Figure 3A) indicate intraalveolar fibrin formation in all groups subjected to fibrin instillation. Because all animals received the same amounts of fibrinogen/thrombin, the extent of fibrin degradation (D-dimers) may be considered as an indication of alveolar fibrinolysis [27]. We cannot exclude that intraalveolar instillation of thrombin caused some alveolar damage. Thrombin might affect the endothelial monolayer and increase permeability, enhancing the formation of edema [28]. However, because all groups received the same amount of thrombin, the observed differences between the groups were caused by the differences in ventilation pressure.

Lung injury

The aim of the present study was to investigate the effects of lung injury on alveolar fibrinolysis. Lung injury was achieved by mechanical ventilation applying high pressure amplitudes, as in previous studies [29,30]. The significant decrease in PaO₂, survival time, P/V plots, and the increase in lung weight per BW in groups with larger pressure amplitudes (groups 2 and 3) clearly document VILI.

Depressed fibrinolytic capacity

Fibrinogen/thrombin instillations resulted in fibrin controls in a measurable fibrin breakdown within 5 min after the instillation procedure without PAI-1 production (Figure 3A). In ventilated animals fibrinolysis was most preserved in animals of group 1 (highest levels of D-dimer and no PAI-1 production). In groups 2 and 3, however, PAI-1 production increased and fibrin breakdown decreased. Further, fibrin breakdown did not depend on the duration of mechanical ventilation (survival time) (Figure 4) but was correlated with the size of the pressure amplitude and the extent of lung injury (Figure 5). With respect to the large amounts of fibrinogen/thrombin which had been instilled, the results of the present study must be interpreted qualitatively and confirm particularly that mechanical ventilation may disturb alveolar fibrin breakdown. From clinical and experimental lung injury studies (e.g., induced by sepsis, pneumonia) we know that intraalveolar fibrinolysis is downregulated due to increased production of PAI-1 [3,6,31]. Indeed, groups 2 and 3 showed increased

PAI-1 levels whereas in group 1 PAI-1 was not detectable (Figure 3B). This demonstrates that aggressive mechanical ventilation with high pressure amplitudes causes PAI-1 production, which downregulates alveolar fibrinolysis. Interestingly, PAI-1 was not detectable in any of the control rats or in rats of group 1 (no increase in lung weight and normal P/V plots). This finding suggests that mechanical ventilation with low pressure amplitude might preserve alveolar fibrinolytic capacity.

Because we studied the effects of mechanical ventilation in previously healthy rats, it is unclear whether there may be a similar PAI-1 production in lungs that have been already injured, for example, by sepsis or pneumonia. The possibility of additive effects on PAI-1 synthesis in preinjured lungs can be expected because elevated PAI-1 levels in BALF from ALI/ARDS lungs [31] and infected lungs [32] have been demonstrated.

The exact mechanisms of PAI-1 production due to mechanical ventilation are not known. From experiments in sepsis or pneumonia-induced ALI it was concluded that PAI-1 related depressed fibrinolysis depends on the interplay between inflammation and coagulation [4,33], with TNF- α as an important link. However, in the present study mechanical ventilation for 225 min triggered TNF- α production in some animals only. No correlation between either TNF- α , PAI-1, or fibrin degradation was found. Our findings reflect the current debate about differences in cytokine response in VILI. Obviously the role of TNF- α and possibly other inflammatory mediators seems to depend on the experimental model applied [30,34]. With respect to our objective, their role of up- or downregulation of ventilation-induced PAI-1 production remains unclear and needs further elucidation.

In this context, other mechanisms, unrelated to inflammatory mediators, may be responsible for mechanical ventilation-associated depressed fibrinolytic capacity [22,35]. The newest concept of VILI considers plasma membrane wounding due to shear stress as the initial trigger for multiple intracellular signal induction pathways (e.g., plasma membrane lipid trafficking, nuclear factor κ B, protein kinase systems) and the synthesis of a variety of mediators and proteases [36,37]. Links to the fibrinolytic system (e.g., PAI-1 production) may be possible, which is supported by new insights from other research areas where cell wounding is considered the major

determinant of PAI-1 mediated fibrin formation. In idiopathic pulmonary fibrosis, alveolar epithelial cell damage and pathological wound repair directly initiate (without inflammation) overexpression of PAI-1 and persistence of fibrin [38]. Furthermore, it has been shown that PAI-1 production is triggered by transforming growth factor- β 1 after mechanical stretch in lung epithelial cells [3,39,40,41]. The fact that cells in general react on mechanical stress with downregulation of the fibrinolytic system has also been demonstrated on vascular endothelial cells, where flow-induced shear stress triggered PAI-1 production [42]. The clinical importance of an intraalveolar antifibrinolytic milieu in mechanically ventilated patients with ALI has recently been underscored by the results of a study in adult patients with ARDS in which high levels of PAI-1 in pulmonary edema fluid were associated with higher mortality [43]. Our findings clearly suggest that mechanical ventilation is one of the factors explaining this latter observation.

CONCLUSION

Alveolar fibrinolytic capacity can be suppressed (PAI-1 dependently) by mechanical ventilation with high pressure amplitudes. This finding may add new information to the pathogenesis of VILI. Our results need to be confirmed by investigations in other lung injury models with intrinsic fibrin formation mimicking diseases such as pneumonia or sepsis.

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ALVEOLAR FIBRINOLYSIS AND MECHANICAL VENTILATION

Part B

Mechanical ventilation affects alveolar fibrinolysis in LPS-induced lung injury

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ABSTRACT

The aim of the present study was to determine the effects of mechanical ventilation on alveolar fibrin turnover in lipopolysaccharide (LPS)-induced lung injury. In a randomised controlled trial, Sprague–Dawley rats (n=61) were allocated to three ventilation groups after intratracheal LPS (*Salmonella enteritidis*) instillations. Group I animals were subjected to 16 cmH₂O positive inspiratory pressure (PIP) and 5 cmH₂O positive end-expiratory pressure (PEEP); group II animals to 26 cmH₂O PIP and 5 cmH₂O PEEP; and group III animals to 35 cmH₂O PIP and 5 cmH₂O PEEP. Control rats (not mechanically ventilated) received LPS. Healthy rats served as a reference group. Levels of thrombin–antithrombin complex (TATc), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen in bronchoalveolar lavage fluid were measured.

LPS-induced lung injury increased TATc, D-dimer and PAI activity and PAI-1 antigen levels versus healthy animals. High pressure-amplitude ventilation increased TATc concentrations. D-dimer concentrations were not significantly raised. Instead, PAI activity increased with the amplitude of the pressure, from 0.7 U/mL in group I to 3.4 U/mL in group II and 5.0 U/mL in group III. There was no change in PAI-1 antigen levels.

In conclusion, mechanical ventilation creates an alveolar/pulmonary anti-fibrinolytic milieu in endotoxin-induced lung injury which, at least in part, might be due to an increase in plasminogen activator inhibitor activity.

INTRODUCTION

intraalveolar fibrin depositions are the pathognomonic hallmark of acute lung injury (ALI) on lung microscopy [1–3]. The fibrin matrix inactivates and incorporates surfactant, leading to severe hypoxaemic respiratory failure [4,5]. Alveolar fibrin is part of the inflammatory response seen in ALI and may initiate fibrotic repair with long-term compromised pulmonary function [6,7]. Intraalveolar fibrin formation in ALI occurs after capillary alveolar leakage of plasma fibrinogen, activation of coagulation and suppression of local fibrinolysis [7–10]. Alveolar macrophages and alveolar epithelial cells are directly stimulated by bacterial endotoxins or indirectly by pro-inflammatory mediators (e.g. tumour necrosis factor (TNF)- α) to produce procoagulant and anti-fibrinolytic proteins. Activated factor VII and tissue factor on the pro-coagulant side, and plasminogen activator inhibitor (PAI)-1 on the anti-fibrinolytic side, are the main mediators of disturbed fibrin turnover. The degree of alveolar fibrin formation and the persistence of fibrin depend mainly on suppressed fibrinolytic capacity due to increased local production of PAI-1 [3,7–9,11–15]. Under normal circumstances, intraalveolar fibrin is resolved within minutes by plasmin [16] and intact surfactant is released, restoring pulmonary function [17]. During ALI, however, alveolar fibrin turnover is disturbed and aggravated by additional insults (e.g. haemorrhagic shock, infections, ventilator-associated pneumonia) [18,19]. The present authors recently demonstrated that injurious mechanical ventilation can depress alveolar fibrinolytic capacity in healthy rats after iatrogenic fibrin formation [20]. In the present study, the effects of different ventilation strategies on alveolar fibrinolysis were examined in rats with “pre-injured” lungs due to endotoxin-induced lung injury.

MATERIALS AND METHODS

The present study was approved by the Animal Committee of the Erasmus University Rotterdam (Rotterdam, The Netherlands). Care and handling of the animals were in accordance with the European Community guidelines. The experiments were performed at the Dept of Anaesthesiology, Erasmus MC-Faculty Rotterdam in male Sprague–Dawley rats (IFFA Credo, Someren, The Netherlands; $n=61$) with a mean $\pm$ SEM body weight of 283.4 ± 2.6 g.

Induction of intraalveolar fibrin formation by local lung inflammation

Lipopolysaccharide (LPS)-induced lung inflammation, adapted from the model originally described by Wheeldon et al. [21] and Van Helden et al. [22], was brought about in 51 animals by intratracheal instillation of 16 mg/kg LPS, derived from *Salmonella enteritidis* (L6761; Sigma-Aldrich, St Louis, MO, USA). The procedure was performed after orotracheal intubation under gaseous anaesthesia (65% nitrous oxide:33% oxygen:2% isoflurane; Pharmchemie, Haarlem, The Netherlands), using a miniature nebuliser (Penn-Century, Philadelphia, PA, USA). After the procedure, rats were extubated.

Experimental protocol

Rats were anaesthetised 24 h after LPS instillation as detailed earlier and tracheotomised. A catheter was inserted into a carotid artery. Anaesthesia was maintained with hourly i.p. injections of pentobarbital sodium (60 mg/kg, Nembutal; Algin BV, Maassluis, The Netherlands). Muscle relaxation was attained with hourly i.m. injections of pancuronium bromide (2 mg/kg, Pavulon; Organon Technika, Boxtel, The Netherlands). After muscle relaxation, all animals were connected to a ventilator (Servo Ventilator 300; Siemens-Elema, Solna, Sweden) set in a pressure-controlled mode with positive inspiratory pressure (PIP) of 12 cmH₂O, positive end-expiratory pressure (PEEP) of 2 cmH₂O, frequency of 30 breaths/min, inspiratory/expiratory time ratio of 1:2 and fractional inspired oxygen tension (FIO₂) of 1.0. Body temperature was kept within normal range by means of a heating pad. After 15 min of stabilisation, arterial blood gases were taken using a carotid artery catheter and PIP was adjusted according to the ventilation group to which the animals had been allocated. Arterial blood gases were sampled every 30 min thereafter using conventional methods (ABL555; Radiometer, Copenhagen, Denmark). Mean arterial blood pressure (MAP) was monitored using the intra-arterial carotid artery catheter every 30 min for 3 h.

Study groups

Animals were allocated to one of three ventilation groups, each with different pressure amplitude. A PEEP of 5 cmH₂O was selected for all groups, but PIP was different in each group. Group I (n=12) was ventilated with a PIP of 16 cm H₂O; group II (n=13) with a PIP of 26 cm H₂O; and group III (n=13) with a PIP of 35 cm H₂O. The ventilation period was designed to last 3 h, after which final measurements were made. FIO₂ was kept at 1.0 throughout the study period to prevent hypoxaemia developing in any of the groups.

Control group animals (n=13) received LPS instillations as described previously ("LPS controls"), but were not mechanically ventilated. To create reference values, a group of healthy rats (n=10) did not receive LPS and were not ventilated ("healthy" animals). At the end of the ventilation period, all rats (n=61) were killed with an intra-arterial overdose of pentobarbital sodium (600 mg/kg) and all measurements were then made.

Bronchoalveolar lavage

After the rats were killed, the thorax and diaphragm were opened and lungs removed. As a parameter of lung injury, subsequently lung weight/body weight ratio was calculated. Bronchoalveolar lavage (BAL) was performed with normal saline (30 mL/kg, heated to 37°C) and re-aspirated three times. BAL fluid (BALF) was centrifuged (400xg for 10 min at 4°C) and the recovered supernatant fluid was snap-frozen and stored at -80°C until further processing. Measurements were not completed in two rats of group II and in three rats of group III, owing to air leakage during the test.

Measurements

Coagulation activation, as assessed by thrombin–antithrombin complexes (TATc), was measured in BALF with an ELISA-based method. Briefly, rabbits were immunised with mouse thrombin or rat antithrombin. Antithrombin antibodies were used as a capture antibody; digoxigenin-conjugated antithrombin antibodies were used as detection antibodies; horseradish peroxidase-labelled sheep anti-digoxigenin antigenbinding fragments (Boehringer Mannheim, Mannheim, Germany) were used

as staining enzyme, and o-phenylenediamine dihydrochloride (Sigma-Aldrich) was used as substrate. Dilutions of mouse serum (Sigma-Aldrich) were used for the standard curve, yielding a lower detection limit of 0.3 ng/mL [23].

Fibrin breakdown of LPS-induced alveolar fibrin formation was determined by measurements of D-dimers (cross-linked fibrin degradation products) in BALF [24]. D-dimers were quantitated by a sandwich-type ELISA (Asserachrom D-dimer; Diagnostica Stago, Asnières-sur-Seine, France). This assay shows cross-reactivity with rat D-dimers.

PAI activity in BALF was determined using an automated coagulation analyser (Behring Coagulation System; Dade Behring, Marburg, Germany) with reagents and protocols from the manufacturer. This assay determines the urokinase-inhibiting activity of the sample. The remaining urokinase is then assayed by activating plasminogen to plasmin and subsequently determining plasmin chromogenic activity. The assay is independent of variable concentrations of plasminogen, α -2-antiplasmin and fibrinogen in the sample. The upper detection limit of this test is set at 6.9 U/mL. Protein concentration in BALF was measured using the Bradford method (Bio-Rad protein assay; Bio-Rad Laboratories, Munich, Germany [25].

To determine the levels of PAI-1 antigen in BALF, a rat PAI-1 ELISA was developed using a rabbit polyclonal antibody (Abcam Ltd, Cambridge, UK) as coating antibody and a biotinylated rabbit immunoglobulin G antibody (Molecular Innovations Inc., Southfield, MI, USA) as developing antibody. Rat PAI-1 (Calbiochem, La Jolla, CA, USA) was used as a standard.

To illustrate alveolar fibrin depositions, samples were taken from all lung lobes and 30 fields were analysed. The analysing pathologist was not informed about the purpose of the study and was asked to prepare rat lungs for illustration of alveolar fibrin deposition. Histological analysis was performed as previously described [20]. Briefly, lungs were fixed at 10 cmH₂O MAP, and slices were stained for fibrinogen. During the washing procedure, fibrinogen would have been washed out leaving solely fibrin attached to the alveolar wall. Slides of lung tissue were deparaffinised and endogenous peroxidase activity was quenched by a solution of methanol/0.03% H₂O₂ (Merck, Darmstadt, Germany). After digestion with a 0.25%

weight/volume solution of pepsin (Sigma-Aldrich) in 0.01 M HCl, the sections were incubated in 10% normal goat serum (Dako, Glostrup, Denmark) and then exposed to biotin-labelled goat anti-human fibrinogen antibody (Accurate Chemical & Scientific Corporation, Westbury, NY, USA). After washes, slides were then incubated in a streptavidin-ABC solution (Dako) and developed using 1% H₂O₂ and 3.39-diaminobenzidin-tetra-hydrochloride (Sigma-Aldrich) in Tris-HCl. The sections were mounted in glycerine gelatin and counterstained with haematoxylin.

Statistical analysis

Data are presented as mean $\pm$ SEM. Group differences were analysed with ANOVA, while differences between the three ventilated groups were analysed using a Kruskal Wallis test. Differences between the healthy and LPS control group, and between the ventilated groups and the LPS control group, were analysed with a Mann Whitney U-test. A p-value <0.05 was considered significant. In Table 1, all values for TATc, D-dimer, PAI activity and PAI-1 antigen were corrected for their individual corresponding protein levels. Analysis was performed as described previously.

RESULTS

All animals survived the study period. LPS instillation caused severe respiratory distress with tachypnoea and significant weight loss, from 283.4 ± 2.6 g before LPS instillation to a mean of 260.3 ± 2.6 g ($p < 0.001$) 24 h after LPS instillation. Mechanical ventilation with high pressure amplitudes caused lung injury with compromised partial pressure of oxygen in arterial blood (PaO₂); group III showed lower PaO₂ values compared with group I at 120, 150 and 180 min (Figure 1; $p < 0.05$). Furthermore, animals in ventilation group II (pre-study MAP 124 ± 6 mmHg versus end-of-study MAP 105 ± 6 mmHg; $p < 0.05$) and ventilation group III (pre-study MAP 126 ± 4 mmHg versus end-of-study MAP 73 ± 5 mmHg, $p < 0.001$), ventilated with high pressure amplitudes, showed lower MAP values at the end of the study period. At the end of the experiment, the extent of lung injury was also evidenced by a significant increase in median lung weight/body weight in group II of 9.2 ± 0.4 g/kg and in group III of 13.3 ± 0.8 g/kg, versus 7.8 ± 0.6 g/kg in animals from group I (table 1).

Figure 1. The effect of mechanical ventilation on partial pressure of oxygen in arterial blood (PaO_2) over time in the three ventilated groups. ●: group I; ■: group II; ▲: group III

*: $p < 0.05$ versus group I. 1 mmHg = 0.133 kPa.

Table 1. Lung weight, total BALF protein levels and values for thrombin-antithrombin complex (TAT), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen adjusted for protein levels in BALF.

	Healthy	LPS controls	Group I	Group II	Group III	P value between ventilated groups
Animals n	8	11	11	12	12	
Lung weight/body weight g/kg	6.0 ± 0.4	8.1 ± 0.3*	7.8 ± 0.6	9.2 ± 0.4	13.3 ± 0.8#	0.01
Total protein mg/mL	0.09 ± 0.01	0.7 ± 0.11*	0.54 ± 0.06	0.67 ± 0.07	1.02 ± 0.12	0.01
TAT/protein ng/mg	42.6 ± 13.7	116 ± 15.8*	85.1 ± 6.6	74.7 ± 6.9	67.6 ± 9.7	0.23
D-dimer/total protein µg/mg	21.8 ± 7.2	22.2 ± 4.2	88.6 ± 35.2#	62.7 ± 7.9#	56.3 ± 13.4	0.76
PAI activity/total protein U/mg	0.0 ± 0.0	0.9 ± 0.6*	1.1 ± 0.7	4.4 ± 0.9#	5.3 ± 0.7#	0.005
PAI-1 antigen/total protein ng/mg	0.3 ± 0.2	19 ± 2.3*	25.3 ± 4.2	24.9 ± 2.6	18.3 ± 3.1	0.23

Data are presented as means ± SEM unless otherwise stated. Adjustments were made for protein content in BALF per individual sample. LPS: lipopolysaccharide.

*: p<0.05 vs. healthy controls.

#: p<0.05 vs. LPS controls.

Figure 2. Effect of mechanical ventilation on thrombin–antithrombin complex (TATc), D-dimer, plasminogen activator inhibitor (PAI) activity and PAI-1 antigen levels in bronchoalveolar lavage fluid in lipopolysaccharide (LPS)-pre-treated animals. *: $p < 0.05$ versus healthy controls; #: $p < 0.05$ versus LPS controls; ¶: $p < 0.05$ between ventilated groups.

Coagulation activation

LPS instillation resulted in activation of coagulation. TATc levels increased from 3.3 ± 1.0 ng/mL in healthy animals to 78.6 ± 15.2 ng/mL in LPS-treated animals (Figure 2). Increased pressure amplitude resulted in a significant increase in TATc levels between the three ventilated groups (Figure 2). Only TATc levels in group I animals were significantly lower compared with LPS controls (Figure 2).

Fibrinolysis

In LPS controls, absolute D-dimer levels in BALF were 13.4 ± 2.0 mg/L, compared with 1.8 ± 0.6 mg/L in healthy rats ($p < 0.001$), indicating alveolar fibrin formation caused by endotoxin (Figure 2). Mechanical ventilation increased D-dimer levels in BALF compared with LPS control animals. Different ventilation pressure amplitudes did not significantly alter D-dimer levels between groups I-III; group I D-dimer levels were 36.1 ± 8.3 mg/L, versus 65.8 ± 16.9 mg/L in group III.

Anti-fibrinolysis

PAI activity was undetectable in BALF of healthy rats and was 1.24 ± 0.8 U/mL in LPS controls (Figure 2). Between the ventilated groups, PAI activity levels were significantly increased; PAI activity concentrations in BALF were 0.7 ± 0.5 U/mL, 3.4 ± 0.8 U/mL and 5.0 ± 0.7 U/mL in groups I, II and III, respectively (Figure 2). PAI activity in group III was also significantly different from that in LPS control animals. Minimal levels of PAI-1 antigen were detected in healthy animals (0.03 ± 0.02 ng/mL), but instillation of LPS led to a significant rise in PAI-1 antigen (13.9 ± 3.6 ng/mL; $p < 0.05$; Figure 2). There was no difference in PAI-1 antigen levels between the three ventilated groups, or between the ventilated groups and the LPS control animals (Figure 2).

Histology

In Figure 3, microscopy of representative rat lung tissue shows lung inflammation caused by LPS. In all ventilated animals, unresolved fibrin depositions (Figure 3c and d) were observed. More fibrin deposits were observed in animals ventilated with the highest pressure amplitude, although there were no differences in levels of lung inflammation. Atelectasis was also more pronounced in animals of group II and especially group III.

Figure 3. Microscopy of representative fibrin-stained paraffin sections of rat lung tissue of lipopolysaccharide (LPS)-treated rats. a) Normal lung tissue; b) lung tissue of a representative sample of the LPS control group (24 h after LPS instillation) predominantly showing interstitial infiltrates with neutrophils; c) representative lung tissue of group III, showing interstitial infiltrates with neutrophils and unresorbed fibrin/fibrinogen depositions (counterstained with haematoxylin) after 3-h mechanical ventilation; and d) a close-up of c), showing unresorbed fibrin/fibrinogen depositions (arrows). Scale bars: 0.1 mm.

DISCUSSION

The present study investigated the effect of mechanical ventilation on alveolar fibrinolysis in LPS-induced lung injury. Intratracheal LPS instillation caused local activation of coagulation, with an increase in TATc levels with fibrin formation (as documented by the appearance of D-dimer in BALF of animals of the LPS control group). The occurrence of alveolar fibrin formation after LPS-induced lung inflammation has been documented previously in a comparable experimental model [24]. Mechanical ventilation aggravates this endotoxin-induced lung inflammation and might therefore influence alveolar fibrin turnover [26]. Therefore, the experimental model of the present study may be considered an appropriate model for the study of the effects of mechanical ventilation on alveolar fibrinolysis.

Acute lung injury is characterised by alveolar flooding. Ventilator-induced lung injury aggravates or may even be the cause of this flooding [27]. In the present study, pulmonary oedema formation was also observed, as characterised by the BALF protein levels and increased lung weights of the animals ventilated with higher pressure amplitudes.

D-dimer concentrations in the BALF of the ventilated animals were higher than in LPS controls. However, there were no significant differences between the ventilation groups. In contrast, PAI activity increased with the size of the pressure amplitude, without a change in PAI-1 antigen levels. The present results link injurious ventilation settings in inflamed lungs with depressed fibrinolysis for the first time. Furthermore, independent of protein leakage into the lung, injurious mechanical ventilation increased the PAI activity in BALF.

The current authors observed an increase in PAI activity but did not see an increase in PAI antigen levels. Conversion of PAI-1 between its active and latent forms is regulated by vitronectin, which circulates in plasma but is also a major constituent of the extracellular matrix [28, 29]. In the data adjusted for total protein levels, an increase in activity without a change in antigen levels was still observed, suggesting that PAI activity is dependent on protein/vitronectin influx and subsequent stabilisation of PAI activity during high pressure amplitude ventilation.

Surprisingly, increased PAI activity did not result in lower amounts of D-dimer in

BALF. There might be two possible explanations. First, 3-h mechanical ventilation might not be enough to demonstrate a larger effect on the downregulation of alveolar fibrin breakdown due to PAI production. Secondly, the high D-dimer levels in the BALF of group III animals (although not significantly different compared with the other ventilated groups, despite high PAI activity) might be explained by additional alveolar fibrin formation, triggered by traumatic mechanical ventilation and an influx of plasma proteins. It is impossible in the current experiment to distinguish whether the increased levels of D-dimers reflect increased fibrinolysis only, or whether they also reflect a higher level of pro-coagulation, resulting in increased formation of fibrin, which will translate into increased D-dimer levels.

The idea of extra fibrin formation in a second-hit model of lung injury (endotoxin plus mechanical ventilation) is plausible. Under both circumstances, similar inflammatory mediators are expressed (e.g. $\text{TNF-}\alpha$), which may activate the intraalveolar coagulation system and fibrin formation [18,30]. LPS induces coagulation activation as indicated by increased TATc levels, whereas mechanical ventilation with low pressure amplitudes significantly reduced this. A possible explanation for this phenomenon is that, with higher pressure amplitudes, resolution of coagulation is impaired. Despite these questions concerning the levels of D-dimer in BALF, the interpretation of the PAI values is striking. Aggressive mechanical ventilation can upregulate PAI, the strongest antifibrinolytic mediator, which might contribute to the persistence of alveolar fibrin and aggravate lung injury (e.g. lung fibrosis). The clinical importance of PAI-1 upregulation in mechanically ventilated patients has only recently been reported [31]. In adult ALI patients, high levels of PAI-1 in pulmonary oedema fluid have been associated with an increase in mortality [31]. The fact that mechanical ventilation may affect the alveolar fibrinolytic milieu has been demonstrated previously [20]. However, in that study, alveolar fibrin formation was generated by instillations of fibrinogen/thrombin in healthy rats, whereas in the present study the amount of alveolar fibrin formation was dependent on the endogenous capability of each animal to build alveolar fibrin after the LPS challenge. A recent report on early stress-response genes demonstrated that coagulation genes are upregulated during ventilation-induced lung injury [32].

The major limitation of the present study is that it was not possible to determine the origin of the fibrin deposits, or more accurately the breakdown product D-dimers, in the BALF. The immunohistochemistry data do not allow the present authors to distinguish between fibrin and fibrinogen in the alveolar, capillary or interstitial space. Despite this, the D-dimer values in BALF clearly demonstrate increased breakdown of intraalveolar fibrin deposits during mechanical ventilation, suggesting increased fibrin deposits in the alveolar space.

In summary, the present study provides new information showing that mechanical ventilation influences alveolar plasminogen activator inhibitor activity after endotoxin exposure and can influence alveolar fibrinolysis. Future experimental studies are needed to elucidate the underlying mechanisms.

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CLINICAL FOLLOW UP

Respiratory sequelae after acute hypoxemic respiratory failure in children with meningococcal septic shock

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ABSTRACT

In an observational follow-up study from 1999 to 2000, we assessed respiratory sequelae in the youngest survivors of meningococcal septic shock (MSS) with acute hypoxic respiratory failure (AHRF). We included children who survived from MSS and AHRF, with a maximum age of five years at follow-up. AHRF was defined based on the first, second and fourth criteria of the American-European Consensus Conference (A-ECC) on the acute respiratory distress syndrome (ARDS).

Twelve children with AHRF were selected. Seven of these children had ARDS. Two children of those with ARDS suffered from respiratory sequelae. The degree of respiratory sequelae was associated with a lower ratio between the pressure of arterial oxygen and the fraction of inspired oxygen, with a higher oxygenation index, with a larger number of ventilation days and a higher lung injury score.

Our observational results suggest that the incidence of long-term respiratory sequelae in children after AHRF appears to be lower than previously reported, but may be higher in those who suffered ARDS. This observation requires further confirmation in future studies within homogeneous and well-defined study populations.

INTRODUCTION

The majority of children with severe meningococcal septic shock (MSS) suffer from acute hypoxic respiratory failure (AHRF) necessitating mechanical ventilation [1]. After recovery from the acute period, patients with sepsis and AHRF are prone to long-term respiratory sequelae. This was reported by small and uncontrolled follow-up studies in 37%-100% of children older than five years [2-5]. However, age might have an impact on the pulmonary healing process, since lung development continues through childhood and may be different when compared to older children and adults [6-8]. In the under five-age group, only passive respiratory function tests under sedation can be performed. This presents a drawback for follow-up studies and explains the fact that no data have yet been published on this subject.

We therefore performed an observational follow-up study in the youngest survivors of MSS with AHRF, who have been considered at highest risk of long-term sequelae.

MATERIALS AND METHODS

Follow-up population

We performed a limited observational follow-up project during 1999-2000, which was approved by our institutional ethics committee. We measured the respiratory function in children under five years old, who had been previously diagnosed with meningococcal septic shock and were being mechanically ventilated on our pediatric intensive care unit (PICU) for AHRF. Our PICU is a 12-bed multidisciplinary unit and part of a tertiary academic referral center. Our objective was to investigate all children with AHRF, as defined, based on the first, second and fourth criteria of the American-European Consensus Conference (A-ECC) recommendations for ARDS (Table 1) [9]. Using these three of the four A-ECC criteria, we wanted to include all patients with AHRF, regardless of whether left ventricular failure (LVF) was present or not (criterion 3, table 1).

Once parents had agreed to the participation of their children in the follow-up examination, they were asked whether their child had ever suffered from any acute chronic respiratory complaint or had used any medication. They were also asked whether there was a family history of asthma or any other medical problems.

Table 1. American-European Consensus Criteria on ARDS [9]

-
1. Acute onset
 2. $\text{PaO}_2/\text{FiO}_2$ ratio < 26.7 kPa
 3. No signs of left atrial hypertension*
 4. New bilateral infiltrates on frontal chest radiograph
-

* In the original recommendation it is mentioned that there have to be no clinical signs of left atrial hypertension and, if measured, pulmonary artery wedge pressure < 18 mmHg (measured by Swan Ganz catheter) [9]. Because Swan Ganz catheters are not routinely used in our PICU, we modified this criterion. We relied on no positive history of cardiac disease and on no clinical signs or echocardiographic evidence suggesting left atrial hypertension (normal left ventricular shortening fraction (SF) of 0.30-0.40, normal left ventricular diameter, no mitral regurgitation and normal sized left atrium) [11,12].

Data collection at the acute episode

Meningococcal disease was clinically diagnosed and proved by positive blood culture. Shock was defined as a systolic blood pressure < 70 mm Hg for children under 12 years old, who were dependent on continuous intravenous catecholamine therapy [10]. On a daily basis, all children with meningococcal septic shock on mechanical ventilation were screened for AHRF based on the A-ECC criteria (Table 1). This included a) calculation of the mean of the lowest ratio between arterial oxygen pressure and inspired oxygen fraction ($\text{PaO}_2/\text{FiO}_2$ ratio) per 24 hours (second A-ECC criterion), b) evaluation of chest radiographs for the presence of bilateral pulmonary infiltrates (fourth A-ECC criterion) and c) three-dimensional echocardiography for measuring left ventricular shortening fraction (SF) and excluding left atrial hypertension (third A-ECC criterion). The echocardiographic criteria have been described previously [11,12].

Severity of disease

The severity of disease at admission was documented by recording the Pediatric Risk of Mortality Score (PRISM II) [13]. To determine the severity of AHRF, we performed the Lung Injury Score (Table 2) [14] and collected the following data: number of ventilation days, daily calculation of the $\text{PaO}_2/\text{FiO}_2$ ratio, oxygenation index ($\text{OI} = \text{Mean airway pressure} \times \text{FiO}_2 \times 100/\text{PaO}_2/7.5$, in kPa, converted to torr).

Respiratory Function Measurements

Before respiratory function tests were started, we measured actual body weight and length. Plethysmographic transcutaneous arterial oxygen saturation (SaO_2) was routinely monitored during the tests (Hewlett Packard Component Monitoring System). Respiratory function measurements were performed during quiet sleep in supine position with the SensorMedics 2600 (Yorba Linda, CA, U.S.A., a pediatric infant lung function measurement device) by experienced staff members. Sleep was induced with the administration of chloral hydrate orally or rectally (50-100 mg/kg), a type of sedation that does not influence gas exchange and does not induce hypoventilation or upper airway obstruction [15]. Flow-volume curves were registered during quiet breathing. We measured the static compliance and resistance of the patient by performing automatic occlusion during end inspiration (measured by the pneumotach) invoking a Breuer-Hering reflex. Pressure at the mouth of the patient was measured with a pressure transducer. We used the open nitrogen (N_2) washout method to measure the functional residual capacity (FRC). If a decreased percentage of time to peak tidal expiratory flow to total expiratory time (% exp. time to PTEF) was observed, bronchodilator therapy with salbutamol inhalation was performed and the effect documented.

Table 2. Lung Injury Score (Murray et al.) [14]

	Value
Chest Roentgenogram Score	
No alveolar consolidation	0
Alveolar consolidation in one quadrant	1
Alveolar consolidation in two quadrants	2
Alveolar consolidation in three quadrants	3
Alveolar consolidation in four quadrants	4
Hypoxemia Score (kPa)	
$\text{PaO}_2/\text{FiO}_2 \geq 40$	0
$\text{PaO}_2/\text{FiO}_2$ 30-39	1
$\text{PaO}_2/\text{FiO}_2$ 25-29	2
$\text{PaO}_2/\text{FiO}_2$ 15-24	3
$\text{PaO}_2/\text{FiO}_2 < 15$	4
Positive End-Expiratory Pressure Score (cm H ₂ O)	
≤ 5	0
6-8	1
9-11	2
12-14	3
≥ 15	4
Final Value *	
No lung injury	0
Mild to moderate lung injury	0.1-2.5
Severe lung injury	> 2.5

* Obtained by dividing the aggregate sum by the number of components that were used.

Calculation of Respiratory Function Parameters

The tidal volume (TV), respiratory rate, percent expiratory time to peak tidal expiratory flow (% exp. Time to P_{tef}), tidal expiratory flow at 25% to P_{tef} (tef₂₅/ P_{tef}) and P_{tef} to tidal volume (P_{tef}/TV) were calculated from the flow-volume curves. The passively exhaled extrapolated volume was divided by the mouth pressure during occlusion, yielding the static compliance. The resistance of the respiratory system was obtained by dividing the time constant by compliance. Flow-volume curves were only accepted for calculation when the flow-volume relationship was linear over at least 50% of the exhaled tidal volume. Compliance (C_{rs}) and resistance (R_{rs}) were calculated as the mean of the data results of 6-16 occlusions. FRC was calculated also as the mean from at least two reproducible measurements. All calculations were corrected for the dead space (V_D) of the respiratory function system. The V_D of the switching valve was 7 ml and of the two types of masks used (50 ml and 25 ml mask) 25 ml and 10 ml, respectively.

Statistical analysis

Statistical analysis was performed using the SPSS software for Windows, version 10.0 (Chicago, Ill. U.S.A.). Normal distribution was visually detected by probability plots of the residuals and tested formally by Kolmogorov-Smirnov test. Patients' baseline characteristics were summarized with descriptive statistics as mean $\pm$ standard deviations. If values were not normally distributed, they were presented as median $\pm$ range. In order to compare the patient's data the Mann Whitney U-test was used. Tests were performed two-sided. Significance was considered if $p < 0.05$.

RESULTS

Characteristics of the patients during the acute episode and at follow-up

In total, 12 survivors met the inclusion criteria for AHRF; all their parents gave consent to participate in the follow-up program. None of the children had an individual or family history of allergy or asthma. They had no respiratory complaints nor were they on any medication. The characteristics of each patient are presented in Tables 3 and 4. Seven children (58%) fulfilled all four criteria for the diagnosis of ARDS (ARDS group).

Clinical symptoms at follow-up

Only one patient (patient C) suffered wheezing within two months after discharge. He was treated with beclomethasone inhalations for 7 months and salbutamol was only used when required. On follow-up, this child was free of symptoms.

Respiratory function measurements

Patients C and D suffered from respiratory function abnormalities (Table 5). In patient C a decrease in % exp. time to PTEF, with decreased PTEF/TV and decreased FRC (suggesting combined obstructive and restrictive pulmonary abnormalities) was observed at six months after discharge. This child also had mild exchange disturbance in measuring SaO_2 of 93% during chloral hydrate sedation. Because of these abnormalities we repeated the measurements 12 months after discharge; at that stage all except SaO_2 had normalized (Table 5). Patient D had a slight decrease in % exp. time to PTEF, indicating only a small degree of obstruction. Because this abnormality was minor we decided not to re-evaluate this child.

Table 3. Patient characteristics during the acute episode

Patients	Group	Age at MSS in months	PRISM	OI (highest) mmHg	Mean lowest PaO ₂ /FiO ₂	Ventilation days	Total Lung Injury score
A	ARDS	29	30	19	15	8	2.6
B	ARDS	17	36	13	15	11	3.0
C ^a	ARDS	13	24	76	7	40	4.0
D	ARDS	23	17	45	8	17	3.3
E	ARDS	14	53	14	25	6	2.0
F	ARDS	31	17	8	20	6	2.6
G	ARDS	35	19	11	26	7	2.0
H	AHRF-LVF	38	12	6	26	6	1.6
I	AHRF-LVF	44	No data	9	17	6	2.0
J	AHRF-LVF	42	15	17	11	7	3.3
K	AHRF-LVF	3	12	4	23	2	1.3
L	AHRF-LVF	11	14	8	24	14	2.0
ARDS group		23 (13-35) ^b	24 (17-53) ^c	14 (8-76) ^b	15 (7-26) ^b	8 (6-40) ^b	2.7 ± 0.8 (1.6-4)
AHRF-LVF group		38 (3-44) ^b	13 (12-15) ^c n=4	8 (4-17) ^b	23 (11-27) ^b	6 (2-14) ^b	2.0 ± 0.8 (1.3-3.3)

OI = Mean airway pressure (MAP) x FiO₂ x 100 / PaO₂ (kPa)/ 7.5 (converted to torr)

^a The acute episode course of ARDS was complicated by air leak syndrome: pneumothorax, pneumomediastinum and pneumopericardium.

^b Values are median (minimum – maximum).

^c Values are mean (±SD)

Table 4. Patient characteristics at follow-up

Patients	Group	Age at follow-up in months	Interval between ARDS and follow-up in months	Height in cm	Weight in kg
A	ARDS	41	12	100	16
B	ARDS	36	17	103	22
C ^a	ARDS	20	6	88	13
D	ARDS	36	13	97	15
E	ARDS	37	23	98	15
F	ARDS	43	12	94	14
G	ARDS	57	22	107	16
H	AHRF-LVF	60	25	115	22
I	AHRF-LVF	50	6	107	16.5
J	AHRF-LVF	48	6	106	17
K	AHRF-LVF	14	11	79	10.4
L	AHRF-LVF	17	6	80	12
ARDS group		37 (20-57) ^b	13 (6-23) ^b	98 (6) ^c	15.9 (2.9) ^c
AHRF-LVF group		48 (14-60) ^b	6 (6-25) ^b	97 (17) ^c	15.8 (4.7) ^c

^a Follow-up data at his first visit, 6 months after discharge^b Values are median (minimum – maximum)^c Values are mean (± SD)

Table 5. Respiratory function measurements of patients C and D

Patients	Group	TV	Resp. rate p. m.	% exp. time to		TEF25/PETF	PTEF/TV	Crs		FRC (N ₂)	SaO ₂
				PTEF					Rrs		
C ⁺	ARDS	9.4 ^a	30 ^a	0.16^a	0.56^a	1.78 ^a	1.04 ^a	0.016 ^a	17.4^a	93%	
		9.7 ^b	30 ^b	0.13^b	0.57^b	1.64 ^b	1.29 ^b	0.014 ^b	17.1^b		
C ⁺⁺	ARDS	9.5 ^a	25 ^a	0.27 ^a	0.72 ^a	1.20 ^a	1.42 ^a	0.023 ^a	24.4 ^a	96%	
		9.2 ^b	22 ^b	0.32 ^b	0.66 ^b	0.95 ^b	1.63 ^b	0.029 ^b	22.3 ^b		
D	ARDS	9.9 ^a	22 ^a	0.07^a	0.66 ^a	0.94 ^a	1.85 ^a	0.029 ^a	24.3 ^a	>97%	
		9.7 ^b	22 ^b	0.10^b	0.51^b	1.19 ^b	1.57 ^b	0.023 ^b	23.9 ^b		
Normal values ^c		7-10	15 - 40	>0.25	>0.6	<1.5	1.0-4.0	<0.05	20-35	>97%	

Definitions of abbreviations: PTEF = Peak tidal expiratory flow (mL/s). TV = Tidal volume (mL/kg). TEF 25 = Tidal expiratory flow when 25% of the TV still has to be expired (mL/s). Crs = Static compliance of the respiratory system (mL/cmH₂O/kg). Rrs = Resistance of the respiratory system (cmH₂O/mL/s). FRC = Functional residual capacity (mL/kg). Obstruction parameter: % exp. time to PTEF = time to peak tidal expiratory flow/ total expiratory time. Restriction parameter: PTEF/TV. **Bold numbers**: abnormal values.

+ First visit: 6 months after discharge; ++ Second visit: 12 months after discharge.

^a Before therapy with bronchodilator.

^b After therapy with bronchodilator.

^c A problem with normal values of lung function parameters is that they show a large natural (inter-subject) variation. If available, the listed normal values of the lung function parameters as in accordance with the European Respiratory Society/American Thoracic Society (ERS/ATS) standards. Otherwise various sources have been used [17,19].

Correlation between symptoms, respiratory function test results and parameters of the acute episode

The child with the poorest respiratory function test was only the child with clinical symptoms at follow-up (patient C). This patient suffered from the most severe form of ARDS, with the longest duration of ventilation days, the highest OI, the lowest $\text{PaO}_2/\text{FIO}_2$ ratio, the highest lung injury score and a serious air leak syndrome complicating the course of the ARDS (Table 3). Furthermore, both children with respiratory function abnormalities (Patients C and D) had ARDS and showed a significantly higher median of duration of ventilation days (29.0 vs. 6.5; $p < 0.030$), higher median OI (60.5 kPa vs. 10.0 kPa; $p < 0.031$), lower median $\text{PaO}_2/\text{FIO}_2$ ratio (7.5 kPa vs. 21.5 kPa; $p < 0.030$) and a higher mean lung injury score (3.7 vs. 2.3; $p < 0.02$) compared to the other ten children with AHRF.

DISCUSSION

In a group of 12 children under five years with MSS and AHRF, we found respiratory sequelae in only two children. This seems to reflect a lower incidence, than the 33%-100% previously reported in older children [2-5]. There may be three reasons for this. Firstly, we had a larger median follow-up interval (12 months) in our patients. Golder et al. found that most of the lung recovery occurs within that period [3], leaving some patients in which the healing process stops at a plateau of lower values for respiratory function parameters [2,3,16]. We were able to confirm this observation in patient C at six and 12 months of follow-up (Table 5), at which stage all parameters except for SaO_2 had normalized. Secondly, we included children with AHRF from one etiology, which was MSS, we defined AHRF uniformly and we focused on one group, which is very different to the previous reports [2-4]. A well-defined study design is strongly recommended in order to increase comparability between studies in AHRF [9,17]. Thirdly, the SensorMedics 2600 machine is unable to measure ventilatory inhomogeneity. More recently advanced respiratory function test machines have become available, which can measure this parameter (e.g., Vmax 26, VIASYS, SensorMedics, Yorba Linda, CA, USA.). They might reveal more abnormalities in children with uncovered compromised respiratory function in future investigations.

Regarding clinical symptoms, wheezing for a period of two months was observed in only one patient. The most frequently reported symptom in literature is also wheezing, but the incidence varied greatly from 0% [4,5] to 100% [2,3]. Remarkably, inhalation therapy with beta-2 adrenergic bronchodilators neither improved the patient's condition nor respiratory function tests [3,4] and this was confirmed by our results (Table 5).

When we looked at the respiratory function abnormalities, patients C and D showed both obstructive and restrictive defects; this is in accordance with previous observations [2-4]. Furthermore, in injured lungs, gas exchange at rest and after exercise can be compromised [2,5,16,18]. In one pediatric study, 78% of the patients had reduced SaO_2 and in another investigation it was found in 33 % (18 children, 3.4 years after meningococcal septic shock) SaO_2 was decreased after exercise, whereas no abnormalities were present at rest [2,5]. We only measured masked gas exchange abnormality ($\text{SaO}_2=93\%$) in one patient during chloral hydrate sedation (Table 5) without any clinical signs of chronic hypoxemia.

We acknowledge three limitations of our observational follow-up study, which are inherently related to the study population and the respiratory function test device (SensorMedics 2600) we used. Firstly, we did not perform any exercise test, gas diffusion test or forced expired maneuver (e.g. "pump and squeeze" method). These techniques make it possible to more accurately determine gas exchange abnormalities, total lung capacity, vital capacity and forced expired volume, unmasking restrictive and obstructive lung diseases which might contribute to a higher incidence of long-term respiratory sequelae [5,15]. However, these techniques are difficult to perform in this very young age group and are accompanied by discomfort for the child recovering from a very traumatic experience. Secondly, we have not been able to recruit an appropriate control group.

Our institutional ethics committee did not agree with the inclusion of a healthy control group due to the sedation protocol. However, sedation of patients in this age group (mean age 36 months) is necessary. Otherwise cooperation of the child is not achieved and respiratory function measurements will not produce reliable and reproducible data. Thirdly, we were unable to recruit enough patients to reach a satisfactory level of statistical significance.

In summary, we have provided the first results of respiratory long-term sequelae of the youngest survivors of AHRF. We have investigated a very homogenous group of patients (all had MSS), which will increase comparability with future studies. Our observations suggest that the incidence may be lower, that recovery may occur more frequently than previously reported, and that children with severe ARDS might be at greater risk. Despite the difficulties involved in performing respiratory function tests in this very young age group, future studies should confirm this observation.

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SUMMARY IN ENGLISH AND DUTCH

Summary

Chapter I presents an introduction to the main aims of the work presented in this thesis. Up to now, almost all scientific data on acute lung injury (ALI) have been derived from experimental studies in adult animals, or from clinical studies in adult patients. In an overview article we describe the current knowledge on ALI with a focus on controlled clinical studies in children.

Four decades of extensive research have revealed that ALI may primarily be regarded as an inflammatory lung disease. After an initial event (e.g. sepsis, pneumonia), activation of a variety of cellular pathways triggers an overwhelming inflammatory response affecting all parts of the lung. The balance between pro-inflammatory and anti-inflammatory mediators is shifted towards inflammation. Inflammation is linked to extravascular/intraalveolar and intravascular activation of coagulation and suppression of fibrinolysis. Endothelial and capillary cell dysfunction causes disturbance of the alveolar-capillary barrier and alveolar flooding with plasma fluids, proteins and cells (e.g. polymorphonuclear leucocytes). Surfactant production and function are diminished. Unresolved fibrin incorporates inactivated surfactant and together they form intraalveolar hyaline membranes. Diffusion of O₂ and CO₂ is hampered, dependent lung regions are largely consolidated, compliance is lost and venovenous mismatch is increased. The accessible area for ventilation and gas exchange is shrunk to the size of a “baby lung”. Clinically, profound hypoxemia and new bilateral infiltrates on chest X-ray are diagnosed.

Epidemiological data on pediatric ALI are still scarce and mainly depend on the country in which (and from how many units) these data have been collected. Despite the inhomogeneity of data on ALI, the incidence and mortality in children is much lower than in adults. However, pediatric mortality is still very high at about 20-30%. It is established that not all patients are susceptible to ALI. It seems that gender-related (male predominance), genetic and ethnic factors may determine which patients will develop ALI and whether or not the course of their disease will be deleterious.

Treatment options are limited and no curative therapy exists. Mechanical ventilation supplying oxygen-enriched gas enables respiration and can be life saving. However, it is known that mechanical ventilation itself can cause certain types of lung injury. Having recognized and acknowledged the main mechanisms leading to ventilator-induced lung injury (VILI), several trials succeeded in convincing physicians to apply a lung-protective ventilation strategy. This new approach of optimal lung recruitment, sufficient positive end-expiratory pressure (PEEP) and small volume ventilation has the aim to minimize VILI and to decrease mortality in adult patients. However, no comparable data exist for pediatric patients with ALI; ventilator settings on the pediatric intensive care unit (PICU) are derived only from experience with this condition in adults.

Many other supportive treatment options have been tried in adults as well as in children (e.g. nitric oxide (INO), high frequency oscillatory ventilation (HFOV), inhaled prostacyclin (PGI₂), prone positioning, surfactant therapy, steroids, fibrinolytic agents, etc.) but without convincing success. Future studies are needed to test their validity.

To recover from ALI, the underlying disease has to be successfully treated. For restoration of normal lung function, epithelial and alveolar cell function with clearance of lung water is necessary. In adults, the quality of life and respiratory function are significantly impaired in many patients with ALI. Follow-up data in children are not comprehensive and have been difficult to collect; nevertheless such data are still urgently needed.

As mentioned above, most data on pediatric ALI have been derived from studies in adult patients. With this study we aimed to gather the available clinical data on pediatric ALI and then supplemented this with data derived from our own clinical research. The results indicated that we needed to perform more basic studies in order to find answers to newly raised questions about (unrecognized) mechanisms that might be important in relation to life-long damage to a child's vulnerable lung.

In **Chapter III** we examined the incidence of ALI in children as well as gender-related differences. On a large multidisciplinary PICU, the incidence of ALI was 9.9% among 35 mechanically ventilated children; almost 80% of this group developed the severe form of ALI, i.e. the acute respiratory distress syndrome (ARDS). With the exception of one child, of the 12 children in this group who died all had ARDS (11/35). As risk factors, we identified that a low average arterial oxygen tension to fraction of inspired oxygen ratio on day one and the severity of multi-organ failure were correlated with mortality. Dependent on the study design and study population, different risk factors have been identified in the past. Most studies confirmed the association between multi-organ failure and mortality; however, the impact of respiratory variables is still a topic of debate.

Lungs of male infants have smaller volumes and airways compared to girls. During critical illness, boys tend to have a worse outcome. Preliminary results from a large European ARDS database suggest that male children < 1 year of age are more susceptible to ARDS than their female counterparts. Whether humoral and/or genetic factors account for these gender-related differences still needs to be determined.

Severe hypoxemia is the main challenge for critical care physicians treating patients with ALI. Therefore, it is assumed that therapies that improve oxygenation will improve the final outcome. In **Chapter IV** we addressed the effect of selective pulmonary vasodilators on oxygenation. In the only randomised controlled trial conducted in children, we found that inhaled PGI₂ at 30 ng/kg/min is able to improve oxygenation by at least 26%. The combination of two pulmonary vasodilators, PGI₂ and INO, may enhance this effect, because they act via two different cellular pathways. Both improve oxygenation by redirecting blood flow from non-ventilated lung areas to ventilated areas, and both lower pulmonary vascular resistance thus easing pulmonary blood flow. However, final outcome parameters, such as mortality, have not yet been examined in children.

During the last decade increasing evidence has emerged from experimental and clinical studies showing that inflammation and coagulation are closely linked during ALI. VILI follows similar -almost identical- pathways to that of the original lung injury, and alveolar fibrin depositions are characteristic features of both ALI and VILI. Therefore, it can be hypothesized that activation of intraalveolar coagulation and inhibition of fibrinolysis are one of the underlying mechanisms of VILI. In **Chapter V** we present the first experimental data derived from a model of rat lung injury. High delta pressure ventilation (35/5 cm H₂O), which is considered to be traumatic to lung tissue, increased intraalveolar fibrin formation in healthy as well as in pre-injured lungs (i.e. endotoxin-induced lung injury). Increased PAI-1 activity creates an intraalveolar antifibrinolytic milieu disturbing fibrin turnover. Persistence of unresolved fibrin leads to alveolar fibrosis, with unknown consequences for the development of the growing lung during childhood. Therefore, based on these preliminary data, the impact of VILI on alveolar/pulmonary coagulation/fibrinolysis should be further investigated in clinical studies. The first results of pilot studies confirm these results. Antifibrinolytic therapy might therefore be an option for ALI and VILI, and has recently been investigated in an experimental setting with success.

The quality of critical care medicine needs to be assessed on both short-term and long-term outcomes. With respect to pediatric ALI and VILI, it is not yet established to what extent the growing lung is damaged beyond recovery, or is able to compensate by ongoing growth. Unfortunately, the performance of respiratory function tests is difficult in pre-school children, albeit essential for follow-up. In **Chapter VI**, we performed elaborate respiratory function testing in a very young age group suffering from ALI caused by a single underlying disease (i.e. meningococcal septic shock). In 2 of 7 children (mean age 37 months) we found respiratory function abnormalities that were partly sub-clinical. No quality-of-life data are yet available for these children but are currently being collected (personal communication).

Concluding remarks

Pediatric ALI has a rare occurrence with an incidence of about 3 cases per year/100,000 persons, and the underlying diseases are very different from those occurring in adults. Mortality is considerable at about 20-30%. The severity of the disease, the degree of hypoxemia and the category of the underlying diseases correlate with the final outcome.

Not all mechanisms related to ALI and VILI have been fully studied. Research on individual susceptibility to develop these syndromes (e.g. genetic polymorphism and the role of apoptosis) has only just started. Similarly, the inter-relation between alveolar inflammation and coagulation/fibrinolysis is well recognized; however, we need more insight into these particular mechanisms.

Based on the results from experimental research we will learn whether new therapies can be added to our treatment protocols, such as, for example, fibrinolytic agents to solve alveolar fibrin depositions, thus restoring function of the alveolar unit.

Despite insufficient data on lung protective strategies in the pediatric age group, most data can be extrapolated from studies in adult patients. Lung protective ventilation may be provided by ventilator settings with sufficient PEEP, low PIP and low VT. Simply put, mechanical ventilation should keep the lungs open and prevent lungs from becoming overdistended. Although clinical data are lacking, there is a strong rationale to combine several interventions which are known to improve the patient's condition: e.g. prone position, INO/PGI₂, surfactant therapy, optimal sedation and nutritional support. In desperate situations, the therapeutic spectrum can be expanded with HFOV and ECMO.

In answer to the question why so many treatment strategies which result in short-term improvements of, for example, oxygenation, do not alter outcome – one can-

not necessarily expect that therapies which, e.g. improve oxygenation, will also improve clinical outcomes. It seems that in many studies there was a failure to identify the most appropriate subset of patients who would most likely respond to treatment.

Furthermore, future clinical trials on pediatric ALI should acknowledge that ALI is a complex and heterogeneous syndrome; this implies that the study design will also not be straightforward. It will be important to enrol those patients at higher risk for adverse clinical outcomes; this group will be more likely to benefit from the new clinical interventions. To find such subsets of patients it is essential to evaluate novel therapies, such as HFOV. One difficulty that still needs to be overcome is that the factors which allow predicting treatment responsiveness are not yet established and are not necessarily intuitive.

Ideally, follow-up should be conducted in all children after suffering critical illness and ALI. Respiratory function measurements as well as quality-of-life evaluation should be performed up until adulthood. Visits should be organized at regular intervals after discharge taking into account the time course of recovery. Factors such as underlying diseases, gender and ethnic background should also be included in the analysis. The results of such follow-up evaluations will then provide intensivists with essential knowledge about the treatment sequelae in their former patients. This is particularly important in the pediatric age group against the background of these children reaching adulthood in an environment of increasing global pollution. This feedback will enable intensivists to reflect on the appropriateness of their therapeutic interventions - still following the imperative *primum nihil nocere* ('first of all, do no harm').

In the scheme of things the children's lobby is miniscule, and due to the low prevalence of pediatric ALI the industrial production of a small series of any device or drug is extremely cost intensive. Therefore, healthcare policymakers should support all

efforts by pediatric intensivists to organize large-scale trials on epidemiology and therapy. Disappointingly, an attempt to gain support from the European Community for the ESPNIC ARDS Database has recently been rejected. When PICUs cooperate on a nationwide basis and (even more important) collaborate internationally, then sufficiently large numbers of patients will be enrolled in meaningful studies yielding meaningful data. Therefore research in children should be supported. These initial costs can then easily be 'earned back' by better outcomes for critically ill children and by preventing lifelong sequelae with related costs for health care, health insurance and society in general.

Samenvatting

Hoofdstuk I geeft een introductie tot het onderwerp van dit proefschrift. Tot op heden zijn de meeste studies met betrekking tot „acute lung injury“ (ALI) bij volwassen patiënten gedaan. In dit hoofdstuk wordt een overzicht gegeven van de meest relevante, gecontroleerde klinische studies betreffende ALI bij kinderen. Uitgebreid wetenschappelijk onderzoek in de afgelopen veertig jaar liet zien dat ALI vooral beschouwd kan worden als een inflammatoire ziekte van de longen. Na een initiële ernstige ziekte (lokaal in de longen bij voorbeeld longontsteking, danwel generaliseerd bij voorbeeld sepsis) wordt op cellulair niveau een forse ontstekingsreactie met productie en activatie van diverse ontstekingsmediatoren veroorzaakt. Er heerst dan ter plaatse een pro-inflammatoir milieu. De inflammatoire mediators communiceren met de extra- en intra-alveolaire stollingscascade, waardoor stollingsactivatie en remming van de fibrinolyse plaatsvindt. De functie van de alveolo-capillaire cellen raakt hierdoor ernstig gestoord, met als resultaat lekkage van plasma-eiwitten en bloedcellen (neutrofiele leukocyten) van het vasculaire naar het intra-alveolaire compartiment. Hierdoor raakt de aanmaak en functie van surfactant ernstig gestoord. De door de stollingsactivatie aanwezige fibrine-neerslag inactiverend surfactant, en samen vormen ze de intra-alveolaire hyaline membranen. Deze leiden tot atelectasen in een groot deel van de longen met afname van de compliantie. Het longvolume wordt te klein voor voldoende ventilatie. In combinatie met reactieve veno-veneuze shunting binnen het pulmonale vaatbed leidt dit tot een ernstig gestoorde gaswisseling (CO_2 en O_2). De clinicus heeft te maken met ernstige zuurstofarmoede en witte infiltraten op de thoraxfoto.

De epidemiologie van ALI bij kinderen is nog onvoldoende onderzocht, en er worden grote verschillen gerapporteerd in de centra en de landen waarin de onderzoeken hebben plaatsgevonden. Voor zover data ter beschikking zijn, bestaat de indruk dat de incidentie van ALI bij kinderen duidelijk lager is dan bij volwassen patiënten. Ook de mortaliteit is lager, ongeveer tussen 20-30%.

Niet iedereen met een hoge kans op ALI ontwikkelt dit ook daadwerkelijk. Mogelijk spelen het geslacht (m.n. jongens) of andere genetische factoren een belangrijke rol bij het uiteindelijk ontwikkelen van ALI.

Op dit moment zijn er weinig echte therapeutische opties. Nog steeds bestaat de enige ondersteunende behandeling uit de applicatie van extra zuurstof met of zonder kunstmatige beademing. Echter, wij hebben gedurende de laatste 20 jaren geleerd dat kunstmatige beademing ook schadelijk kan zijn (ventilator-induced lung injury (VILI)). Nadat de hoofdverantwoordelijke mechanismen, die VILI veroorzaken, bekend werden, vond men een beademingsvorm die minder traumatisch is. Feitelijk bestaat deze uit een optimaal 'lung recruitment', voldoende positieve eind-expiratoire beademingsdruk (PEEP) en ventilatie met een laag teugvolume (Vt). Met deze beademingsvorm nam de mortaliteit bij volwassen patiënten met ALI af. Echter, er zijn geen onderzoeken, die deze strategie bij kinderen bevestigen.

Verder heeft men naar andere ondersteunende therapieën gezocht (b.v. nitric oxide (INO), hoog frequente oscillerende beademing (HFOV), inhalatie prostacyline (PGI₂), buikligging, surfactant-, corticosteroïd- en fibrinolytische therapie, etc.). Echter, geen van deze therapieën liet in gecontroleerde studies afname van de sterfte zien. Desondanks bestaat de indruk dat toekomstige studies met een beter studieontwerp deze veelbelovende therapievormen opnieuw moeten gaan toetsen.

De voorwaarde om van ALI te herstellen is dat de onderliggende ziekte herstelt. Pas daarna kan ook de beschadigde capillo-alveolaire celfunctie zich herstellen. Want eerst moeten het door de lekkage intra-alveolair terecht gekomen water, eiwitten en cellen worden opgeruimd. Pas dan kan de longfunctie tot de oude prestatie regenereren.

Echter, in sommige patiënten vindt dit herstel niet of maar gedeeltelijk plaats. Bij volwassenen zien wij klinisch respiratoire restverschijnselen en abnormale longfunctie. Ook hun resterende levenskwaliteit is minder. Pilot studies bij kinderen bevestigen deze resultaten. Er ontbreken nog valide follow-up onderzoeken m.n. bij kinderen in hoeverre dit herstel plaatsvindt en het is nog te vroeg voor een definitieve conclusie. Dat was ook de reden waarom wij op basis van onze klinische ervaringen en studies in het laboratorium terecht kwamen. Wij wilden door dierexperimentele onderzoeken inzicht in nieuwe pathofysiologische mechanismen van ALI en VILI krijgen, die misschien voor de kwetsbare longen van een groeiend kind van belang zouden kunnen zijn.

In **hoofdstuk III** tonen wij de resultaten van onze epidemiologische onderzoeken. Wij bepaalden de incidentie en de uitkomst van kinderen met ALI op onze multidisciplinaire kinder intensive care voor kinderen gedurende 2 jaar. Wij vonden 35 kinderen met ALI (9% van alle beademde kinderen), van wie 80% een acute respiratory distress syndrome (ARDS) ontwikkelden, de ernstige vorm van ALI. Twaalf kinderen overleden, waarvan 11 een ARDS hadden. De risicofactoren waren een lage zuurstofspanning in het bloed in verhouding tot de zuurstofconcentratie in de beademingslucht op dag 1 van de beademing, en een hoge score betreffend het falen van de vitale organen. De meeste studies vonden in het verleden dezelfde risicofactoren. Maar er is nog veel discussie over het onderlinge gewicht van deze en andere risicofactoren en hun definitieve betekenis.

Er blijken verschillen tussen mannen en vrouwen te zijn. In het algemeen verlopen levensbedreigende ziekten ernstiger bij jongens dan bij meisjes, en zijn de longen van mannelijke pasgeborenen kleiner dan die van vrouwelijke. In een onderzoek, gebaseerd op een grote Europese database, toonden wij aan dat mannelijke patiënten < 1 jaar leeftijd vaker ARDS krijgen dan meisjes van dezelfde leeftijd. Of humorele of genetische factoren dit verschil kunnen verklaren is nog niet duidelijk.

De ernstige hypoxemie gedurende ALI is het grootste probleem. Daarom is men bezig steeds alternatieve behandelingen te vinden om de zuurstofopname in deze situatie te verbeteren.

Om deze reden presenteren wij in **hoofdstuk IV** een onderzoek, waarin we het effect van inhalatie met epoprostenol op de zuurstofopname bij kinderen met ALI hebben onderzocht. Bij een gemiddelde dosering van 30 ng/kg/min zagen wij een verbetering van gemiddeld 26%. De combinatie van twee verschillende selectieve pulmonale vasodilatoren (PG₂ en INO) kan tot verdere verbetering van de oxygenatie leiden. Desondanks heeft nog geen enkele studie kunnen aantonen dat deze verbetering ook tot een kleinere sterfte leidt.

In de laatste jaren heeft wetenschappelijk onderzoek aangetoond dat er een nauwe relatie bestaat tussen inflammatie en stolling/fibrinolyse. Dit geldt ook voor ALI en VILI, waarin als eindproduct van dit mechanisme intra-alveolaire fibrinedeposities in hyaline membranen kunnen worden aangetoond. In **hoofdstuk V** presenteren wij

voor de eerste keer twee dierexperimentele onderzoeken, waarin de effecten van agressieve beademingsvoorwaarden op de alveolaire fibrinolyse zijn onderzocht. Het bleek dat een hoge peak inspiratoire druk (PIP) van 35 cm H₂O bij een PEEP van 5 cm H₂O tot remming van de alveolaire fibrinolyse leidt. Verantwoordelijk hiervoor is de verhoogde activiteit van plasminogen activator inhibitor type 1 in broncho-alveolaire lavagevloeistof. Door remming van de fibrinolyse worden de intra-alveolaire fibrinemembranen niet opgelost, en wordt de alveolaire fibrose geïnitieerd c.q. gecontinueerd. Deze observatie zou kunnen betekenen dat kunstmatige beademing via de remming van de alveolaire fibrinolyse tot longfibrose en beperkte longfunctie bij kinderen leidt. Ook biedt deze bevinding nieuwe aanknopingspunten voor fibrinolytische therapieën, zoals pilot studies konden aantonen.

In **hoofdstuk VI** vroegen wij ons af wat de beperkingen van de longfunctie van kinderen met ALI op de lange termijn zouden kunnen zijn. Er bestaan helaas onvoldoende studies over follow-up bij kinderen met ALI. In een pilot onderzoek onderzochten wij 12 kinderen < 5 jaar met ALI o.b.v. meningococcensepsis. In deze kleine, maar zeer homogene subgroep van kinderen met ALI vonden wij relevante afwijkingen in longfunctie. Vaak waren deze afwijkingen zonder klinisch correlaat, waardoor ze voor de clinicus moeilijk vast te stellen zijn. Verder zijn er nog geen onderzoeken over de kwaliteit van leven bij deze kinderen gepubliceerd, maar de eerste onderzoeken zijn net begonnen (persoonlijke mededeling).

Conclusie

ALI komt bij kinderen niet vaak op. In een bevolking van 100.000 treedt ALI slechts bij 3 kinderen per jaar op. Dat is veel minder dan bij volwassenen. Ook de frequentie van de onderliggende ziektebeelden verschillen tussen kinderen en volwassenen. De sterfte ligt rond 20-30% en wordt door de agressiviteit van de onderliggende ziekte, de ernst van de oxygenatiestoornis en het aantal falende organen bepaald. Nog niet alle mechanismen van ALI en VILI zijn onderzocht. Tegenwoordig zijn onderzoeken naar genetische oorzaken en de samenhang met apoptosis veelbelovend. Ook de invloed van kunstmatige beademing op de intra-alveolaire stolling/fibrinolyse en de klinische consequenties voor de patiënt moeten verder worden onderzocht.

We mogen als resultaat van deze onderzoeken nieuwe therapieën verwachten, die het beloop van ALI zullen inkorten en afzwaken, en die VILI kunnen voorkómen (b.v. fibrinolyse).

De techniek van de kunstmatige beademing bij kinderen wordt afgeleid van klinisch wetenschappelijk onderzoek bij volwassenen. Daarom gelden bij kinderen ook de principes van long-beschermende beademingsvoorwaarden (optimale PEEP, lage PIP, laag V_t). Hoewel graad I-bewijs ontbreekt, heeft vooral de combinatie van meerdere therapieën (buikligging, PGI_2/INO , surfactant, voeding etc.) een kans de uitkomst van de patiënten te verbeteren. HFOV en ECMO staan gereed voor situaties waarin standaard therapieën falen.

Wat betreft ontwerp van toekomstige studies is het belangrijk de geschikte studiepopulatie te selecteren. Alleen dan zal een uitspraak over het slagen of falen van een nieuwe therapie mogelijk zijn. Zo moeten er criteria worden gedefinieerd, waarmee risicopatiënten op een mogelijk slechte uitkomst worden herkend. Zo'n subgroep maakt meer kans te profiteren van nieuwe therapieën.

Idealiter zou van elk kind in het vervolg van een opname op een intensive care afdeling onderzocht moeten worden, of op lange termijn de levenskwaliteit door de voormalige ziekte wordt beperkt. Met betrekking tot ALI staan hierbij regelmatige longfunctietests tot aan de volwassen leeftijd op de voorgrond. De resultaten moeten worden gecorreleerd aan de onderliggende ziekte die ALI heeft veroorzaakt, het geslacht van het kind en de etnische afkomst. Alleen dankzij deze gegevens krijgt de intensivist voldoende inzicht in de kwaliteit van de intensive care therapie gedurende de acute fase. Het principe van „*primum nihil nocere*“ staat hierbij boven aan. Kinderen hebben niet de sterkste lobby. De medische behandeling van kinderen is in verhouding duurder dan die van volwassenen. De productie van medische apparatuur is bij voorbeeld duurder vanwege de kleine productieaantallen. Daarom is het belangrijk dat gezondheidsmanagers en politici kinderarts-intensivisten ondersteunen in hun inspanningen met behulp van grote internationale multicenter studies relevante nieuwe therapieën te ontwikkelen. De kosten zijn snel terugverdiend als hierdoor levenslange nadelige gevolgen voor de gezondheid van deze kinderen kunnen worden voorkomen.

Curriculum vitae

Peter Dahlem was born on November 26th, 1960 in St. Ingbert, Germany. After completing high school in 1979, he studied philosophy and chemistry for one year at the University of Freiburg, Germany. In 1980 he started medical school at the same university, from which he graduated as medical doctor in 1987. In 1989 he completed the degree as medical doctor at the University of Freiburg with a study on *“Ethnic-historical knowledge about pregnancy and child bearing in the Hotzenwald”*. From 1988 until 1995 he received his pediatric training at the Children’s Hospital in Konstanz, Germany, at the University of Zürich, Department of Neonatology in the Women’s Hospital, Switzerland and at Children’s Hospital, Lörrach, Germany. In 1995 he started as fellow of pediatric intensive care at the Emma Children’s Hospital, Academic Medical Center (AMC) of the University of Amsterdam, the Netherlands. In 1998 he joined the medical staff of the department of pediatric intensive care at the Emma Children’s Hospital, AMC Amsterdam. During this period he focused on children with acute lung injury, and initiated studies for this thesis.

Since 2004 Peter Dahlem is medical director of the Children’s Hospital in Coburg, Germany. He is married to Pia-Maria Dahlem and they are very happy with their recently born first child.

Curriculum vitae

Peter Dahlem werd op 26 november 1960 in St. Ingbert in het zuid-westen van het toen nog gedeelde Duitsland geboren. Na zijn eindexamen van de middelbare school in 1979 studeerde hij gedurende één jaar filosofie en scheikunde aan de Universiteit van Freiburg. Daarno koos hij voor de opleiding geneeskunde in Freiburg, waar hij in 1987 het doctoraalexamen in geneeskunde haalde. Hij volgde tot 1995 de opleiding tot kinderarts in Konstanz, Zürich en Lörrach. In 1989 promoveerde hij aan de Universiteit van Freiburg met een onderzoek naar *“Etno-historisch onderzoek naar zwangerschap en bevalling in het Hotzenwald”*.

In 1995 begon hij een fellowship intensive care voor kinderen in het Emma Kinderziekenhuis /AMC van de Universiteit van Amsterdam. Vanaf 1998 werkte hij aldaar als staflid tot 2004. In die periode vormde kinderen met longfalen zijn interessegebied, en initieerde hij de studies die onderdeel van dit proefschrift zijn. In 2004 accepteerde hij een functie als medisch hoofd van een algemene kinderafdeling in Coburg, Duitsland.

Hij is getrouwd met Pia-Maria Dahlem, en ze zijn gelukkig met hun onlangs geboren eerste baby.

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Prof. Lachmann

Lieber Herr Lachmann. Het was altijd bijzonder plezierig om in uw laboratorium te werken.

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Artsen van de kinder IC

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Laraine Visser, secretaresse van Prof. Lachmann

Dear Laraine, Thanks for your help to make my Dutchified Germanish English real English.

Heleen Thygesen en Marcel Dijkgraaf, klinische epidemiologe

Beste Heleen en Marcel, dank voor jullie statistische hulp.

Joost Meijers en Marcus Schultz

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Clementine Bouwma

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ohne eure unvoreingenommene Unterstützung während der Studienjahre bis hin zum heutigen Tag hätte ich nicht das erreichen können, worauf ich heute mit Stolz zurückblicken darf. Ich war mir eurer Liebe immer sicher.

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ich bin glücklich, mit dir mein Leben teilen zu dürfen. Hierfür reicht Dank nicht aus, trotzdem: Von ganzen Herzen danke, dass es dich gibt und danke für all die Geduld und Unterstützung!

Stellingen behorend bij het proefschrift

Alles wat is ontstaan is afkomstig uit 'Het Ene' (Grieks: $\tau\omicron\epsilon\nu$, Plato 427-347 v. Chr, Athene). Het is goed noch slecht; het is wat het is.

Het groeiende lichaam van een kind verschilt van een volwassen lichaam wat betreft proporties, fysiologie en dynamiek van alle processen.

Per jaar lijden ongeveer 4 kinderen op een populatie van 100.000 mensen aan 'acute lung injury'. *(Dit proefschrift)*.

'Acute lung injury' bij kleine kinderen heeft een ernstiger verloop bij jongens dan bij meisjes. *(Dit proefschrift)*.

Het onderliggende lijden en de mate van longfalen bij 'acute lung injury' bepalen de uitkomst van een kind. *(Dit proefschrift)*.

Naast kunstmatig beademen bestaan er aanvullende therapieën (bijv. selectieve pulmonale vasodilatatie) om de zuurstofopname door de longen te verbeteren. *(Dit proefschrift)*.

In de kinderleeftijd kunnen groeiende longen door ernstige en chronische ziekten, maar ook door de kunstmatige beademing beschadigd worden, hetgeen vergelijkbaar is met een groeiende boom die gesnoeid wordt. *(Dit proefschrift)*.

De door beademing veroorzaakte longschade wordt mede geïnduceerd door remming van de alveolaire fibrinolyse. *(Dit proefschrift)*.

De aanhouder wint, en het komt ooit allemaal goed: "(...) het begin van een opwaartse trend. Niet alleen denken Nederlanders genuanceerder over het oorlogsverleden, ze denken bovenal positief over het karakter van Duitsers." *(www.kennislink.nl 6-9-2006)*.

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