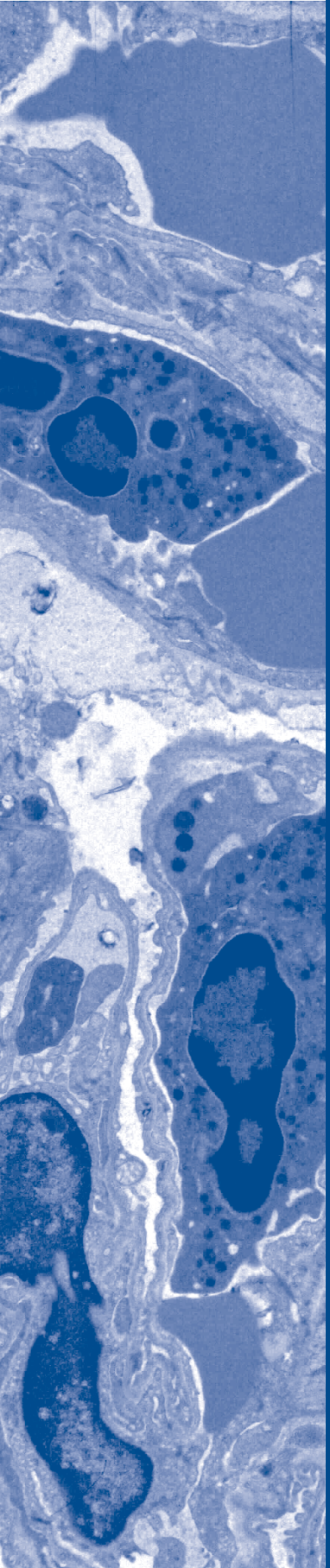

Ventilator Induced Lung Injury in Children in the Intensive Care

Experimental and Clinical Studies

F.J.J. Halbertsma

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Contents

Chapter 1

Introduction and outline of the thesis

Introduction

Mechanical ventilation is a potentially life-saving therapy in the Pediatric Intensive Care Unit (PICU). However, mechanical ventilation also has serious adverse effects on both the lung and other organs. Mechanical ventilation induced lung stretch may result in cellular membrane and tissue damage ("baro-volutrauma"), and activate a local inflammatory response which may spread towards the systemic circulation ("biotrauma"). Experimental research and clinical studies in critically ill adults have resulted in new ventilator strategies that have shown to reduce these side effects. Low tidal volume ventilation resulting in low end-inspiratory alveolar pressures, is the mainstay of this so-called "*lung protective ventilation strategy*".

Despite the fact that the application of low tidal volumes has contributed to lower morbidity and mortality in adults, the fundamental underlying mechanisms are not fully understood, nor has lung protective ventilation strategy been studied in the pediatric population. Children have essential differences in lung anatomy and physiology including maturation of the inflammatory system and age-dependent anatomic and physiologic changes. This implies that the mechanisms of lung injury in relation to various ventilation strategies should be studied in the specific pediatric population.

Outline of this thesis

This thesis investigates various aspects of the effects and pathophysiologic mechanisms of low tidal volume ventilation on the mechanical ventilation-induced inflammatory response and *Ventilator Induced (or Associated) Lung Injury* (VILI / VALI). Both experimental and clinical studies are presented. The thesis is therefore divided in two parts, each starting with a review on the specific subject.

Part I, containing experimental studies, starts with *Chapter 2*. This chapter reviews experimental and clinical studies on mechanical ventilation-induced inflammation and *Ventilator Induced Lung Injury*. Leukocytes and cytokines appear to be important in the pathogenesis of *Ventilator Induced Lung Injury*. There appears to be a distinctive relation between the tidal volume, the magnitude of the inflammatory response, and the histopathological damage.

Chapter 3 describes a new *in-vivo* healthy mouse model developed to study the effects of mechanotransduction. Our specific question was if in the absence of structural lung damage, mechanical ventilation would still induce a pulmonary and systemic inflammatory response.

In *Chapter 4* we studied the effects of hypercapnia on the mechanical ventilation-induced inflammatory response in an experimental *in-vivo* model. By increasing inspiratory CO_2 concentration without changing tidal volume we were able to differentiate between a direct effect of hypercapnia and effects attributable to a decrease in tidal volume.

Part II contains clinical studies in ventilated critically ill children, and starts with *Chapter 5*. This chapter reviews clinical and experimental studies on recruitment maneuvers. These maneuvers, consisting of temporary pressure increase, are often performed to compensate for the possible increase in alveolar collapse caused by a reduction in tidal volume.

In *Chapter 6* we present a survey including all neonatal and pediatric ICUs in the Netherlands concerning the clinical practice of recruitment maneuvers. We were specifically interested in the indications, recruitment mode, applied pressures, and observed adverse effects of recruitment maneuvers.

In *Chapter 7* we describe the effects of a single recruitment maneuver in children with acute lung injury. Besides it effects on oxygenation and ventilation, we analyzed pulmonary and systemic cytokine levels, as we hypothesized that the recruitment maneuver could result in an enhanced local inflammatory response and translocation of inflammatory mediators into the systemic circulation.

In *Chapter 8* we analyzed the effects of several ventilator settings in a cohort of children with normal gas exchange on admission. Our purpose was to answer the question whether a higher tidal volume or PEEP level were associated with increased development of acute lung injury.

In *Chapter 9* a summary of the studies with their conclusion is presented, followed by a general discussion on how these studies may be incorporated in current ventilation practice.

Section I

Experimental studies

Chapter 2

Cytokines and biotrauma in ventilator-induced lung injury: *a critical review of the literature*

F.J.J. Halbertsma, M. Vaneker, G.J. Scheffer, J.G. van der Hoeven.

Netherlands Journal of Medicine 2005; 63:382-92

Abstract

Background: Mechanical ventilation is known to induce and aggravate lung injury. One of the underlying mechanisms is biotrauma, an inflammatory response in which cytokines play a crucial role.

Objective: To review the literature on the role of cytokines in ventilator-induced lung injury (VILI) and multiple organ dysfunction syndrome (MODS).

Material and methods: 57 English written, peer-reviewed articles on cytokines in *in-vitro* settings (n = 5), *ex-vivo* models (n = 9) *in-vivo* models (n = 19) and clinical trials (n = 24).

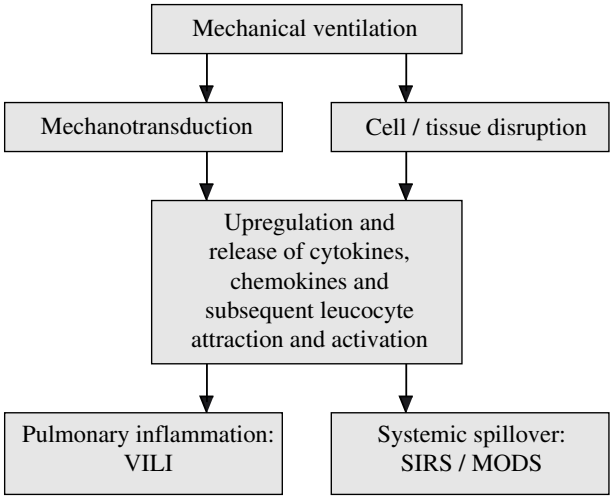
Results: Mechanical ventilation (MV) can induce cytokine upregulation in both healthy and injured lungs. The underlying mechanisms include alveolar cellular responses to stretch with subsequent decompartimentalisation due to concomitant cellular barrier damage. The cytokines involved are interleukin (IL)-8 and CXC chemokines, and probably IL-6, IL-1β, and tumour necrosis factor (TNF)-α. Cytokines are important for signalling between inflammatory cells and recruiting leucocytes to the lung. There is strong circumstantial evidence that the release of cytokines into the systemic circulation contributes to the pathogenesis of MODS. Multiple studies demonstrate the relation between elevated proinflammatory cytokine concentrations and mortality.

Conclusion: Cytokines are likely to play a role in the various interrelated processes that lead to VILI and other MV-related complications, such as MODS and possibly ventilatorassociated pneumonia. Cytokines are good surrogate endpoints in exploring the pathogenesis and pathophysiology of VILI in both experimental and clinical studies.

Introduction

Mechanical ventilation (MV) is one of the cornerstones of ICU treatment. Despite its lifesaving effects, MV may lead to serious damage in both previously healthy and diseased lungs, a process called ventilator-induced lung injury (VILI); (Figure 1). In 1974, Webb en Tiernay demonstrated that MV with high peak airway pressures resulted in lung oedema, alveolar disruption, capillary leakage and death.[1] Further studies revealed that the end-inspiratory volume and not the end-inspiratory pressure was the main determinant (volutrauma). Subsequent studies showed that cyclic opening and collapse of alveoli, even at low inspiratory pressures and low inspiratory volume, increases stretch and shear forces resulting in lung injury and surfactant dysfunction.[2,3] This atelectrauma could be attenuated by increasing positive end-expiratory pressure (PEEP) and outweighed the concomitant increase in inspiratory pressure.[1,4] Recent studies have shown that MV upregulates pulmonary cytokine production, which may result in an in.ammatory reaction aggravating lung injury (biotrauma). This inflammatory reaction is not confined to the lungs but also involves the systemic circulation and has its effects on distal end-organs, which offers an explanation for the observation that most adult respiratory distress syndrome (ARDS) patients do not die from respiratory failure but from multiple organ dysfunction syndrome (MODS).[5] In this review we will discuss the role of cytokines in VILI and relate these findings to the clinical setting.

Figure 1. *Presumed mechanism in VILI and MODS*



VILI = ventilator-induced lung injury; SIRS = systemic inflammatory response syndrome; MODS = mutiple organ dysfunction syndrome

Inflammatory response to mechanical ventilation

Pulmonary injury and inflammation is a complex process in which cytokines play an important role. Cytokines are low-molecular-weight soluble proteins that transmit signals between the cells involved in the inflammatory response.[6] They are produced by bronchial, bronchiolar and alveolar epithelial cells[7] but also by alveolar macrophages and neutrophils.[8] The balance between the proinflammatory cytokines tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-8 and anti-inflammatory cytokines such as IL-10 is essential for directing the immune response.[9] Some of the cytokines have natural antagonists, for example IL-1ra which makes an interpretation of the net effect cumbersome.[10,11] TNF-α and IL-1 induce NF-κB activation, a critical step in the transcription of genes necessary to perpetuate the innate immune response that ultimately results in activation and extravasation of polymorphonuclear leucocytes (PMNs) and other immune active cells, a process that starts within minutes after commencing mechanical ventilation.[12] Leucocytes are predominantly activated and attracted to the lungs by CXC chemokines and IL-8.[13] However, alveolar recruitment of PMNs by instilling a chemoattractant (LTB4) does not result in lung injury,[14] indicating that other factors, possibly cytokines, are necessary to activate them. This activation and attraction of leucocytes is a very important feature in biotrauma. Experimental studies using PMN-depleted animals demonstrate a significantly reduced degree of VILI.[15] Also, leucocyte apoptosis appears to be delayed in adult acute lung injury (ALI) and neonatal chronic lung disease (CLD) [16,17] contrary to pulmonary epithelial cells[18,19] and other end-organs that exhibit increased apoptosis.[20] Incubation of normal PMNs in bronchoalveolar lavage (BAL) fluid derived from ARDS patients results in delayed apoptosis compared with those incubated in normal BAL fluid.[21] Inhibition of neutrophil apoptosis seems mediated by soluble factors, such as the proinflammatory cytokines, possibly IL-8 and IL-2,[22] granulocyte colony-stimulating factor and granulocyte / macrophage colony-stimulating factor (GM-CSF), and levels of soluble Fas-ligand appear to be higher in BAL fluid derived from ARDS nonsurvivors than in that of survivors.[21] Similarly, Fas, Fas-ligand and Caspase-3 are more prevalent in alveolar walls of patients succumbing to ARDS than in those who died without this diagnosis, and soluble recombinant human Fas ligand infusion in the experimental setting results in increased alveolar apoptosis and injury.[23] Another important pathophysiological relation in VILI is that between cytokines and surfactant. Surfactant dysfunction or deficiency is one of the prominent features of lung injury. Inflammation and more specifically cytokines such as TNF-α and IL-1 are thought to decrease surfactant components either directly [24] or indirectly by inducing alveolar leakage of proteins that subsequently inhibit surfactant function.[25] There are several

mechanisms by which mediator release may occur during mechanical ventilation: alterations in cytoskeletal structure without ultrastructural damage (mechanotransduction); stress failure of the alveolar barrier (decompartimentalisation), stress failure of the plasma membrane (necrosis), and effects on the vasculature independent of stretch or rupture.

Mechanotransduction

One of the most intriguing mechanisms of ventilationinduced cytokine release is mechanotransduction. Transmembrane receptors such as integrins, stretch-activated ion channels and the cytoskeleton itself are identified as the key structures in mechanosensing that start various intracellular processes.[26,27] Mechanotransduction, the stimulation of gene transcription following mechanosensing, is most likely signalled by mitogen-activated protein kinase (MAPK).[28,29] Most alveolar cells are capable of producing pro- and anti-inflammatory mediators such as TNF-α, IL-1β, IL-6, IL-8, and IL-10 [8,28,30-34] when stretched *in vitro* [8,32,33,35] or when ventilated with a large tidal volume (Vt) in *ex-vivo* and *in-vivo* experiments (Tables 1 and 3). In premature neonates, cytokine production appears to be related to gestational age, with a delayed maturation of the anti-inflammatory response.[36] Injurious MV also induces upregulation of genes responsible for c-fos which in turn activates transcription for cytokine synthesis,[35] cyclo-oxygenase production and intercellular adhesion molecule (ICAM)-1 expression.[35] NF-κB, a DNA-binding protein, plays a central role as a common messenger in cytokine regulation and inflammation. In experimental models, blockage of NF-κB decreases VILI.[8,37-40] However, its exact role in mechanotransduction is not completely clear yet.[27]

Translocation and decompartimentalisation

Besides mechanotransduction, direct trauma to the plasma membrane of alveolar cells and loss of cell integrity leads to the release of intracellular cytokines to the interstitium and decompartimentalisation into both the alveolar space and the systemic circulation.[41] Experiments by Haitsma et al. have demonstrated that in healthy animals ventilated without positive end-expiratory pressure (PEEP), endotracheal instillation of lipopolysaccharide (LPS) to induce local TNF-α production results in elevated serum concentrations of TNF-α, and conversely intraperitoneal LPS injection resulted in TNF-α in BAL fluid.[42]

Table 1. *Experimental in-vitro studies*

Author, reference	Study subject	Study design	Studied variables	Results
Pugin[8]	Human alveolar macrophages	A: Static B: Cyclic stretch C: LPS static D: LPS + cyclic stretch	TNF-α, IL-6, IL-8 NF-κB activation	IL-8: A < C < B < D TNF-α, IL6: A/B = 0 C < D Dexamethasone blocks increase of TNF-α, NF-κB
Vlahakis[33]	Human alveolar epithelium	A: Cyclic stretch B: Static stretch	IL-8	A > B
Blahnik[36]	Neonatal lung macrophages	LPS stimulation of lung macrophages: A: preterm B: term	TNF-α, IL-10	TNF-α: A = B IL-10: A < B
Li[85]	Neonatal lung macrophages	rIL-10 / dexamethasone administration	IL-6, TNF-α	Decrease
Mourgeon[32]	Foetal rat lung cells	Stretch 0 - 5% ± LPS	MIP-2	Increase with higher stretch levels especially after LPS
Grembowicz[35]	Bovine endothelium	Stretch	c-fos, NF-κB	Increase after plasma membrane disruption

Cytokines in VILI

Experimental studies

Experimental studies consist of both *in-vitro*, *ex-vivo* and *in-vivo* models, using different species and applying various techniques, which probably explains some of the observed inconsistencies in cytokine response (Tables 1 to 3).[43] In almost all studies, cyclic overstretch increases alveolar levels of IL-8 or its rodent equivalent macrophage inflammatory protein (MIP)-2. MIP-2 is the most potent leucocyte chemoattractant and its role in the pathogenesis of VILI is very important. Neutrophil depletion attenuates the increase of IL-8 in the lungs and results in less severe VILI.[15,38] Activation of neutrophils in VILI occurs primarily in the alveolar space after migration. Subsequent lung damage is partly mediated by the interaction of the CXC chemokine receptor 2 ligand in lung tissue with its receptor on neutrophils.[44] Other proinflammatory

Table 2. *Experimental ex-vivo studies*

Author, reference	Study subject	Study design	Studied variables	Results
Tremblay[31]	Isolated rat lung, n = 55	A: MV Vt 7 PEEP 3 B: MV Vt 15 PEEP 10 C: MV Vt 15 PEEP 0 D: MV Vt 40 PEEP 0 NaCl 0.9% vs LPS	TNF-α, IL-β, IFN-γ, IL-6 / 10, MIP-2, c-fos mRNA in BAL	A < B < C < D TNF-α / MIP2 / c-fos: LPS > NaCl 0.9%
Tremblay[7]	Isolated rat lung, n = 24	A: MV Vt 7 PEEP 3 B: MV Vt 15 PEEP 10 C: MV Vt 15 PEEP 0 D: MV Vt 40 PEEP 0 NaCl *vs* LPS	TNF-α, IL-6, mRNA, in lung homogenate, BAL	C and D > A Time-dependent response, peak at T = 30 min
Whitehead[86]	Isolated rat lung, n = 70	A: MV Vt 7 PEEP 3 B: MV Vt 15 PEEP 3 C: MV Vt 15 PEEP 0 D: MV Vt 40 PEEP 0 NaCl *vs* LPS	TNF-α, IL-β, MIP-2, in BAL	NaCl*:* TNF-α, IL-β: A < D LPS*:* TNF-α, MIP-2 A > D
Chu[87]	Isolated rat lung, n = 88	A: MV Vt 7 PEEP 5 B: MV Vt 7 PEEP 0 C: MV Vt 0 PEEP 0 D: MV PIP 50 PEEP 8 E: MV Vt 0 PEEP 50 F: MV Vt 0 PEEP 31	TNF-α, IL-6, MIP-2, in BAL	TNF-α: B > C = A; D = E > F IL-6: B > C = A; D > F = E MIP-2: B > C = A; D = E = F
Ricard[88]	Isolated rat lung, n = 38	A: MV Vt 42 B: MV Vt 7 C: CPAP ± LPS	TNF-α, IL-1β, MIP-2, in serum and BAL	Before LPS: Serum: *A,B* - BAL: MIP-2 / IL-1β: A > B = C TNF-α: - After LPS: Serum: TNF-α, IL-1β, MIP-2: increase B, C, D BAL: TNF-α, IL-1β, MIP-2: B = C > D
Bethmann[34]	Isolated mouse lung, n = 27	A: MV ΔP 10 B: MV ΔP 25 Positive or negative pressure MV	TNF-α, IL-6, mRNA	A < B in both positive and negative pressure ventilation
Cheng[89]	Isolated mouse lung, n = 30	A: MV Vt 7 ZEEP 0 B: MV Vt 7 NEEP -7.5 C: MV Vt 7 NEEP -15	TNF-α, MIP-1, lung dynamics	C > A / B C < B / A
Bailey[48]	Isolated mouse lung, n = 106	A: FiO_2 0.21 B: FiO_2 1.0 ± MV Vt 20	TNF-α, IL-6 in BAL	TNF-α: B + MV > B - MV IL-6: B > A ± MV
Held[40]	Isolated mouse lung, n = 31	A: MV Vt 9 ΔP 10 B: MV Vt 32 ΔP 25 C: LPS	MIP-2, MIP-1α, NF-κB in BAL and Serum	BAL / serum: B = C > A Attenuation by dexamethasone

Table 3. *Experimental in-vivo studies*

Author, reference	Study subject	Study design	Studied variables	Results
Wilson[90]	Mouse, n = 29	A: MV Vt 9 B: MV Vt 35	TNF-α, MIP-2 in BAL	A < B
Wilson[46]	Mouse, n = 15	A: MV Vt 10 B: MV Vt 44 TNF receptor knock out Anti-TNF e.t. wild mice Anti-TNF i.v. wild mice	MIP-2 in BAL Pulmonary PMN influx Lung injury	A < B in all mice PMN influx less in knockout and anti-TNF e.t. mice, not in anti-TNF i.v. mice
Belperio[44]	Mouse, n = 30	A: MV PIP 20 B: MV PIP 40 C57B6 vs CXCR2-/-	KC / CXCL1, MIP-2 / CXCL2/3 in lung tissue	A < B Less in CXCR2-/- mice
Gurkan[91]	Rat, n = 26	A: MV Vt 6 B: MV Vt 17 NaCl 0.9 vs HCL e.t.	Il-6, TNF-α, VEGF in BAL	NaCl: A = B = 0 HCl: IL-6, VEGF: A < B
Chiumello[64]	Rat, n = 40	A: MV Vt 16 PEEP 0 B: MV Vt 16 PEEP 5 C: MV Vt 9 PEEP 0 D: MV Vt 9 PEEP 5 E: MV Vt 9 PEEP 5 + RM HCl e.t.	TNF-α, MIP-2 in serum and BAL	BAL TNF-α: A > D > B > E Serum TNF-α: A > B = D = E BAL MIP-2: A > B = D = E Serum MIP: A > B > D = E
Caruso[92]	Rat, n = 30	A: spontaneous ventilation B: MV Vt 6 C: MV Vt 24	IL-1β mRNA in lung tissue L infiltration	A < B = C
Copland[93]	Rat, n = nd	MV Vt 25 PEEP 0	HSP-70, IL-1β in lung tissue	Increase after 90 min MV
Copland[94]	Rat, n = 18	A: MV Vt 25 B: MV Vt 40 Adult vs neonatal rats	mRNA IL-1β, IL-6, IL-10, TNF-α, MIP-2 in lung tissue	A > B adult > neonatal
Imanaka[95]	Rat, n = 23	A: MV PIP 45 PEEP 0 B: MV PIP 7 PEEP 0	TNF-α mRNA, TGF-β1 mRNA PMN ICAM PaO_2	No increase A = B A < B B < A
Verbrugge[96]	Rat, n = 200	Lung lavage model A: MV + Surfactant B: Partial liquid vent. C: MV PEEP 16 D: MV PEEP 8 E: MV PIP 32/6	TNF-α, protein in BAL	TNF-α: A = B = C = D = E Protein: A = B = C < D = E
Quinn[97]	Rat, n = 35	A: MV FiO_2 0.21 B: MV FiO_2 1.0	MIP-2, WBC in BAL Lung weight	B > A B > A

Table 3. *Continued*

Author, reference	Study subject	Study design	Studied variables	Results
Bueno[98]	Rat, n = 33	A: Vt 7 B: Vt 21 C: Vt 42	TNF-α in plasma PaO_2, lung weight	C > A / B (ns) PaO_2: C < A / B Lung weight: A / B < C
Haitsma[99]	Rat, n = 85	A: MV P 13 / 3 B: MV P 32 / 6 C: MV P 32 / 0	IL-6, MIP-2 in BAL and serum	A / B / C: increase MIP-2 in BAL B / C: increase MIP-2 in serum C: increase IL-6 in serum
Haitsma[41]	Rat, n = 85	A: MV P 45 / 0 B: MV P 45 / 10 LPS et / IP vs NaCl	TNF-α in serum and BAL	A > B LPS > NaCl
Lin[76]	Rat, n = 50	A: MV Vt 7 PEEP 5 1hour/day B: MV Vt 21 PEEP 0 1h/day Bacterial installation e.t.	MIP-2, TNF-α Blood cultures	A > B A < B positive
Herera[100]	Rat, n = 125	A: MV Vt 6 B: MV Vt 20 PEEP vs ZEEP	IL-1β, IL-6, TNF-α serum, mRNA in lung tissue	B ZEEP > A ZEEP > A PEEP
Takata[101]	Rabbits, n = 13	MV P 28 / 5	TNF-α mRNA in lung lavage cells	Increase
Imai[47]	Rabbits, n = 25	A: MV Anti-TNF-α e.t. B: MV IgG e.t. C: MV NaCl e.t.	WBC in BAL	A < B = C
Narimanbekov[45]	Rabbits	A: FiO_2 0.21 low PIP B: FiO_2 1.0 high PIP C: B + rIL-1 antagonist	WBC in BAL	A, C < B

cytokines such as IL-1β and IL-6 are elevated in most but not all studies. Recombinant IL-1 receptor antagonist attenuates neutrophil recruitment in a lung lavage model.[45] The involvement of another potent proinflammatory cytokine TNF-α in the pathogenesis of VILI is still under debate. Increased TNF-α levels after MV were found in most but not all uninjured lung models, surfactant depletion and ALI models, and sepsis models (Tables 2 and 3). Endotracheal instillation of anti-TNF-α antibody attenuates VILI in both the previously uninjured and injured lung, suggesting a role for TNF-α.[46,47] However, lack of TNF-α signalling (TNF-α receptor -/- mice) does not show diminished VILI.[46] In general, most of the reviewed studies show a more pronounced increase in cytokine levels with larger tidal volumes or absent PEEP or when animals

Table 4. *Human studies*

Author, reference	Study subject	Study design	Studied variables	Results
Ranieri[52]	ARDS, n=44	A: Vt 11 PEEP 6.5 B: Vt 7.5 PEEP 14.8	TNF-α, IL-1β, IL-6, IL-8, IL1-RA, in BAL / serum	Most variables A > B
Stuber[50]	ALI, n=12	A1: Vt 5 PEEP 15 (6H) A2: Vt 12 PEEP 5 (6H) A3: Vt 5 PEEP 15 (6H)	TNF-α, IL-1β, IL-6, IL-10, IL1-RA, in BAL / serum	Serum A1 < A3 > A2 BAL A1 < A2 < A3
Wrigge[53]	Elective surgery, n = 39	A: Vt 15 PEEP 0 B: Vt 6 PEEP 0 C: Vt 6 PEEP 10	TNF-α, IL-6, IL-10, IL1-RA	A = B = C
Wrigge[54]	Thoracotomy/ laparotomy, n = 34/30	A: Vt 12 - 15 PEEP 0 B: Vt 6 PEEP 10	TNF-α, IL-1, 6, 10, 12	A = B = C
ARDS network[51]	ARDS, n = 861	A: Vt 6 B: Vt 12	IL-6 Mortality	A < B A < B
Meduri[60]	ARDS, n = 27	A: survivors B: nonsurvivors	TNF-α, IL-1β, IL-6, IL-8	A < B
Meduri[102]	Persistent ARDS, n = 17	A: R/methylprednisolone B: R / -	TNF-α, IL-1β, IL-6 IL-10 mRNA in cells primed with plasma	A < B A > B
Headley[73]	ARDS, n = 43	A: survivors B: nonsurvivors	TNF-α, IL-1β, IL-6, IL-8	A < B
Douzinas[63]	Sepsis / ARDS, n = 8	Mechanical ventilation	TNF-α, IL-6	Arterial > venous
Park[9]	ARDS, n = 69	A: patients at risk for ARDS B: patients developing ARDS	TNF-α, TNF-α R I & II, IL-1β, IL1-RA, sol IL-1β r II, IL-6, sol IL-6 r, IL-8	Anti-inflammatory cytokines / pro-inflammatory cytokines A > B, both > 1
Parsons[70]	ALI, n = 861	A: Vt 6 B: Vt 12	IL-6, IL-8, IL-10	IL-6, IL-8 : A < B Mortality and morbidity related with IL-6, IL-8
Parsons[71]	ALI, n = 95	A: Vt 6 B: Vt 12	Sol TNF receptor I	A < B
Plotz[49]	Infants, n = 12	Vt 10 PEEP 4 Anaesthesia for cardiac catheterisation	TNF-α, IL-6	Increased after 2 hours
Yoon[103]	Neonates, n = 69	Intrauterine infection	IL-6, CLD	IL-6 associated with CLD

Table 4. *Continued*

Author, reference	Study subject	Study design	Studied variables	Results
Wang[104]	Neonates, n = 34	Mechanical ventilation	IL-16 in BAL	Detectable Associated with increased BAL Leucocytes
Kwong[105]	Premature neonates, n = 15	Mechanical ventilation	IL-1β, IL-8, IL-10 in BAL	IL-10 undetectable IL-10 inhibits IL-1β, IL-8 in BAL derived macrophages
Mc Colm[106]	Preterm neonates, n = 17	Mechanical ventilation	IL-1β, IL-8, IL-10 in BAL	IL-10 detectable in CLD, elevated IL-1β, IL-8
Oei[59]	Neonates, n = 48	Mechanical ventilation	IL-10 in BAL	IL-10 increases with GA Low IL-10 in CLD
Schultz[107]	Neonates, n = 20	RDS	IL-10 in BAL	Elevated pro-inflammatory cytokines, stable IL-10
Groneck[55]	Neonates, n = 59	Follow-up infants with prolonged MV need	IL-8 in BAL	Increased IL-8 levels
Hitti[56]	Neonates, n = 136	A: RDS B: no RDS	TNF-α in BAL	A > B
Jonsson[57]	Neonates, n = 28	A: CLD B: no CLD	IL-1β, IL-6, IL-8 in BAL	A > B
Munshi[58]	Neonates, n = 56	A: RDS progress to BPD B: RDS resolving	IL-6, IL-8 in BAL	A > B

are concomitantly subjected to other injurious strategies such as hyperoxia.[48] The observed proinflammatory response usually parallels the observed histopathology. The injured lung appears to be far more susceptible for VILI than the healthy lung (two-hit model).

Human studies (Table 4)

Both short-term and long-term clinical studies have shown that ventilator settings in.uence pulmonary cytokine levels. Plotz et al. demonstrated that two hours of lungprotective MV (Vt 10 ml/kg, 4 cm H_2O PEEP, FiO_2 0.4) in healthy infants anaesthetised for cardiac catheterisation resulted in elevated alveolar IL-6 levels.[49] Stuber et al. showed that increasing Vt from 6 to 12 ml/kg in ARDS patients increases cytokine levels in both BAL fluid and plasma within one hour.[50,51] These findings are consistent with both the results of Ranieri et al. who found lower cytokine levels in BAL fluid of patients ventilated with low Vt[52] and those of the ARDS network trial in

2000 that found lower plasma IL-6 levels in the low Vt group.[51] In accordance with experimental data, previously injured lungs may be more susceptible for VILI. Wrigge et al. found elevated cytokine levels after elective surgery in patients with normal lungs, but there was no difference between patients ventilated with Vt 15 ml/kg and those with Vt 6 ml/kg.[53,54] In longitudinal studies in both adults and neonates,[55-59] elevated proinflammatory cytokine levels are associated with more severe lung injury and worse outcome, supporting the concept that lung injury is partly the result of a massive proinflammatory response.[60-62]

Cytokines and multiple organ dysfunction syndrome

In patients with ARDS the highest cytokine concentrations are found downstream from the lung.[63] Thus biotrauma is not only confined to the lungs but may also result in a systemic inflammatory response syndrome (SIRS) [52,61,62,64] and distant organ apoptosis,[20] both leading to MODS and death. This offers an explanation for the observation that most patients with ARDS do not die from respiratory failure but from MODS.[5] The presumed causal relation between a ventilation-induced increase in systemic cytokine levels and subsequent MODS is an interesting hypothesis.[37,61,62,65-69] Several studies have found plasma cytokine levels to be higher during large tidal volume ventilation [51,52,70,71] and associated with the development of MODS,[72] and persistent cytokine elevation in turn is associated with a poor outcome in patients with ARDS.[60,73] Another important mechanism contributing to the development of MODS is the ventilation-induced enhancement of local dissemination of bacteria[74] and decompartimentalisation of bacteria and endotoxins from the alveolar space into the circulation.[75-77] Bacteria derived from BAL fluid from ARDS patients with persistent local inflammation exhibit enhanced growth capacity when incubated with proinflammatory cytokines.[78] Kanangat et al. showed that the induction of cytokines by LPS diminished the bacterial killing capacity of monocytes.[79] This supports the theory that a persistent local proinflammatory reaction may be a risk factor for developing a ventilator-associated pneumonia (VAP).[80] *In-vitro* corticosteroids block these increased bacterial growth capacities in the presence of high proinflammatory cytokine concentrations.[81] If clinically confirmed this may be an interesting new strategy in preventing VAP in certain selected patient groups. The role of immunomodulation on the clinical course of VILI and MODS needs further investigation. In neonatal RDS, early treatment with corticosteroids has significantly decreased the inflammatory response,[82] diminished CLD and dramatically improved survival, the contribution of corticosteroids in (late) adult ARDS is still controversial.[83]

Conclusions

There is a growing body of evidence that mechanical ventilation may sensitise the innate immune system and that in turn the innate immune system may sensitise the lungs to the effects of mechanical ventilation. This explains the exaggerated ventilation-induced inflammatory response in preinjured lungs and is of great clinical importance.[84] Cytokines play an important role in the various interrelated processes that lead to ventilator-induced lung injury and other related systemic complications, such as multiple organ dysfunction syndrome and possibly ventilator associated pneumonia.

Abbrevations

ALI	actual lung injury
ARDS	adult respiratory distress syndrome
BAL	bronchoalveolar lavage
BPD	bronchopulmonary dysplasia
CLD	chronic lung disease
CPAP	continuous positive airway pressure
e.t.	endotracheal
FiO_2	fractional inspired oxygen
HSP	heat shock protein
ICAM	intercellular adhesion molecule
IL	interleukin
i.v.	intravenous
LPS	lipopolysaccharide
MIP	macrophage inflammatory protein
MV	mechanical ventilation
NEEP	negative end-expiratory pressure (cm H_2O)
PaO_2	pulmonary artery oxygen
PEEP	positive end-expiratory pressure (cm H_2O)
PIP	peak inspiratory pressure (cm H_2O)
ΔP	PIP-PEEP difference
PMN	polymorphonuclear leucocytes
RA	receptor antagonist
RDS	respiratory distress syndrome
rIL	recombinant interleukin
RM	recruitment maneuver
SOL	soluble

TNF	tumour necrosis factor
VEGF	vascular endothelial growth factor
Vt	tidal volume (ml/kg)
WBC	white blood cells
ZEEP	zero end-expiratory pressure

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Chapter 3

Mechanical ventilation in healthy mice induces reversible pulmonary and systemic cytokine elevation with preserved alveolar integrity: *an in-vivo model using clinical relevant ventilation settings*

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Abstract

Background: Mechanical ventilation (MV) may activate the innate immune system, causing the release of cytokines. The resulting proinflammatory state is a risk factor for ventilatorinduced lung injury. Cytokine increase results from direct cellular injury but may also result from cyclic stretch alone as demonstrated *in-vitro*: mechanotransduction. To study mechanotransduction *in-vivo*, the authors used an animal MV model with clinically relevant ventilator settings, avoiding alveolar damage.

Methods: Healthy C57BL6 mice (n = 82) were ventilated (tidal volume, 8 ml/kg; positive end-expiratory pressure, 4 cm H_2O; fraction of inspired oxygen, 0.4) for 30, 60, 120, and 240 min. Assigned animals were allowed to recover for 2 days after MV. Both pulmonary tissue and plasma interleukin (IL)-1α, IL-1β, tumor necrosis factor α, IL-6, IL-10, and keratinocyte-derived chemokine levels were measured. Histopathologic appearance of lung tissue was analyzed by light microscopy and electron microscopy.

Results: In lung tissue, all measured cytokines and keratinocyte- derived chemokine levels increased progressively with MV duration. Light microscopy showed increased leukocyte influx but no signs of alveolar leakage or albumin deposition. Electron microscopy revealed intact epithelial cell and basement membranes with sporadically minimal signs of partial endothelial detachment. In plasma, increased levels of IL-1α, tumor necrosis factor α, IL-6, and keratinocyte-derived chemokine were measured after MV. In the recovery animals, cytokine levels had normalized and no histologic alterations could be found.

Conclusions: Mechanical ventilation induces reversible cytokine increase and leukocyte influx with preserved tissue integrity. This model offers opportunities to study the pathophysiologic mechanisms behind ventilator-induced lung injury and the contribution of MV to the “multiple-hit” concept.

Introduction

Mechanical ventilation (MV) is widely used in general anesthesia and is a lifesaving intervention in critically ill patients. It can, however, induce lung injury in the healthy lung or exacerbate damage in the already injured lung. This has been termed *ventilator-induced lung injury* (VILI).[1,2] Clinical studies show that the use of large tidal volumes (Vt ≥ 12 - 15 ml/kg) is associated with a poor prognosis; however, a "lung-protective ventilation strategy" (low tidal volumes [Vt < 10 - 12 ml/kg], optimizing positive end-expiratory pressure [PEEP]) reduces but cannot prevent VILI.[1,3-8]

Ventilator-induced lung injury is characterized by the release of inflammatory mediators (especially cytokines), infiltration of leukocytes, alveolar and interstitial edema, alveolar protein deposition, cellular necrosis, and tissue disruption.[9,10] It is now commonly accepted that increased production of cytokines, especially interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, plays a key role in initiating or perpetuating lung injury.[11-17]

The clinical relevance of cytokine up-regulation by MV is the resulting proinflammatory state, because this makes the host more vulnerable to a "second hit" (e.g., major surgery).[18] To note, MV itself can be the "second hit" where an already comprised host exists (e.g., MV in the critically ill patient).[3,4,19]

Two mechanisms are believed to be responsible for MV-induced cytokine release. The first is direct trauma to the cell with disruption of the membranes, resulting in translocation of cytokines into both the alveolar space and the systemic circulation. This "decompartmentalization" has been demonstrated *in-vivo*.[13,20,21] The second has been termed *mechanotransduction*. *In-vitro* studies show that most pulmonary cells, such as alveolar macrophages, epithelial cells, and endothelial cells, can produce cytokines in response to cyclic stretch.[22-24] However, the sensing mechanism of these physical forces and the translation into intracellular signals is largely unknown.[25]

In many of the currently available experimental VILI models, injurious MV settings (e.g., Vt > 25 ml/kg or peak pressures > 20 - 40 cm H_2O) have been used in healthy animals,[11,12,14,26,27] or the "multiple-hit" model was used by applying MV in already injured animals.[13,15,16,28,29] From these study designs, it is not possible to differentiate whether the observed increase in cytokine levels is the result of decompartmentalization, mechanotransduction, or both. For a better understanding of the relevant pathophysiologic mechanisms leading to VILI, it is important to study *in-vivo* the effects of ventilation in the healthy lung, using ventilatory protocols analogous to those currently used during general anesthesia and in the intensive care unit patient. We studied the effects of MV in healthy mice, carefully searched for pulmonary cell or tissue disruption, counted leukocyte numbers, and measured cytokine production in lung tissue and plasma.

Materials and Methods

All experiments were approved by the Regional Animal Ethics Committee in Nijmegen, The Netherlands, and performed under the guidelines of the Dutch Council for Animal Care and the National Institutes of Health.

Animals

Experiments were performed in male C57BL6 mice (n = 82; Charles River, Sulzfeld, Germany) aged 10 - 12 weeks, with weights ranging from 23 to 28 g.

Mechanical ventilation in mice

Mice were anesthetized with an intraperitoneal injection of a combination of ketamine, medetomidine, and atropine (KMA): 7.5 µl per gram of body weight of induction KMA mix (consisting of 1.26 ml ketamine, 100 mg/ml; 0.2 ml medetomidine, 1 mg/ml; 1 ml atropine, 0.5 mg/ml; and 5 ml NaCl, 0.9%) was given just before intubation. Animals were orally intubated under direct vision with an endotracheal tube (0.82 mm ID, 1.1 mm OD, length 25 mm). Endotracheal tube position was confirmed by end-tidal carbon dioxide analysis, using mass spectrometry. Subsequently animals were connected to the ventilator (MiniVent®; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Vt was set at 8 ml/kg and frequency was set at 150/min, which is well within the range of measured Vt and respiratory rate during spontaneous ventilation in C57BL6 mice.[30] All animals received 4 cm H_2O PEEP. To avoid direct oxygen toxicity, as reported by several authors,[31,32] the fraction of inspired oxygen (FIO_2) was set at 0.4.

To maintain anesthesia, 5.0 µl per gram of body weight boluses of maintenance KMA mix (consisting of 0.72 ml ketamine, 100 mg/ml; 0.08 ml medetomidine, 1 mg/ml; 0.3 ml atropine, 0.5 mg/ml; and 18.9 ml NaCl, 0.9%) were given, *via* an intraperitoneally placed catheter, every 30 min. Throughout the experiment, rectal temperature was monitored and maintained between 36° and 37.5° C using a heating pad.

Study groups

Animals were divided into seven groups. Group C (n = 9) served as control group: After induction of anesthesia, these mice were killed immediately, without being ventilated. Animals in groups 30 (n = 6), 60 (n = 6), 120 (n = 9), and 240 (n = 9) were ventilated for 30, 60, 120, and 240 min, respectively, and were killed immediately thereafter. In group R (recovery group), animals (n = 6) were extubated after being ventilated for 240 min and were killed after 2 days of recovery. Anesthesia was discontinued in the group R animals 1 hour before extubation. Group D (depleted

group) animals (n = 6) were first leukocyte depleted by administering cyclophosphamide as described previously.[33,34] These animals were then ventilated for 240 min and killed immediately thereafter.

A separate set of experiments (intra-arterial blood pressure [IABP] group, n = 15) was conducted to assess whether the chosen anesthetic and ventilation regime resulted in a stable and reproducible cardiorespiratory condition. In these animals, continuous intra-arterial carotid blood pressure was measured. Arterial blood gas analysis was performed after 120 min (n = 6) and 240 min (n = 9). The same ventilator settings were used as for the mice in the aforementioned groups. We decided not to include the animals from the IABP group for the cytokine or histopathologic analysis to avoid possible interference with cytokine response resulting from instrumentation induced tissue damage.

In addition, two control experiments were performed. In the first control experiment, animals (n = 12) received the standard ventilation strategy (Vt, 8 ml/kg; PEEP, 4 cm H_2O; FiO_2, 0.4). The lungs were removed after 0 min (n = 4, control), 120 min (n = 4), and 240 min (n = 4) of MV to measure wet/dry ratios. In the second control experiment, animals (n = 4) were ventilated with a Vt of 16 ml/kg, PEEP of 4 cm H_2O, and FIO_2 of 0.4 for 240 min. The lungs of these mice were used to histopathologically assess the effects of high-Vt ventilation in our model.

Material harvesting

After the animals were killed, blood was collected by exsanguination and centrifuged at 14,000 rpm (13000 g) (Eppendorf 5415 C; Nethler-Hinz GmbH, Hamburg, Germany) for 2 min, and plasma was stored at -80° C. Immediately after exsanguination, the heart and lungs were carefully removed *en block* via midline sternotomy. The right middle lobe was fixed for light microscopy (LM) and electron microscopy (EM), except in animals analyzed for wet/dry ratio. The remaining lung tissue was homogenized for the determination of cytokine concentrations.

Preparation and analysis of lung tissue

For LM, the material was fixed in 4% buffered formalin solution overnight at room temperature, dehydrated, and embedded in paraplast (Amstelstad, Amsterdam, The Netherlands). Sections of 4 μm thickness were used for further analysis. The enzyme activity of leukocytes was visualized by enzyme histochemistry using chloracetatesterase staining (Leder). Periodic acid-Schiff staining was performed to analyze for alveolar albumin presence. Leukocytes were counted manually (20 fields per mouse), and after automated correction for air/tissue ratio, leukocytes per μm^2 were calculated.

For EM, the material was fixed in 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate buffer, pH 7.4, overnight at 4° C and washed in the same buffer. The tissue fragments were postfixed in cacodylate-buffered 1% OsO_4 for 120 min, dehydrated, and embedded in Epon 812 (Merck, Darmstadt, Germany). Ultrathin sections were cut on an Ultratome (Leica, Reichert Ultracuts, Vienna, Austria), and contrasted with 4% uranyl acetate for 45 min and subsequently with lead citrate for 4 min at room temperature. Sections were examined in a Jeol 1200 EX2 electron microscope (JEOL, Tokyo, Japan). The evaluating pathologist was blinded to the group and ventilation protocol to which the animal had been assigned.

For wet/dry ratios, both lungs were used; ratios were calculated by measuring lung weight before and after heating for 24 hours in a stove at 50° C.

Laboratory tests

Interleukin-1α and IL-1β were assessed using specific radioimmunoassays, as described previously.[35] Levels of TNF-α, IL-6, IL-10, and keratinocyte-derived chemokine (KC) in lung homogenate and plasma were measured using enzyme-linked immunosorbent assay (for TNF-α, IL-6, and IL-10: CytoSet, BioSource, Camarillo, CA; for KC: ELISA-Kit, R&D Systems, Minneapolis, MN). Lower detection limits were as follows: IL-1α and IL-1β: 40 pg/ml; TNF-α: 32 pg/ml; IL-6: 160 pg/ml; IL-10: 16 pg/ml; and KC: 160 pg/ml. For the assessment of KC in plasma in group 60, insufficient plasma was available; the plasma had to be diluted for analysis, which increased the detection limit to 1600 pg/ml. To investigate whether lipopolysaccharide contamination was present in our experimental setting, we measured lipopolysaccharide in air, tubing, and the ventilator by Limulus Amebocyte Lysate testing (Cambrex Bio Science, Walkersville, MD; detection limit: 0.06 U/ml).

Statistical analysis

Data are expressed as mean (SD) when distributed normally (leukocyte counts and wet/dry ratios) and expressed as median (range) otherwise (cytokine concentrations). Statistical analysis was performed with SAS (SAS Institute Inc., Cary, NC) statistical procedures. Because cytokine concentrations are not normally distributed, Kruskal-Wallis procedures were used, with post hoc comparisons of subgroups (Duncan). Data of a particular cytokine concentration variable were ranked, followed by analysis of variance in the General Linear Models procedure using the MEANS procedure with the Duncan option and Bonferroni correction for multiple comparisons. For the analysis of leukocyte counts and wet/dry ratios, analysis of variance was used on nonranked data with *post hoc* comparison of group means (Duncan). The level of significance was set at $p < 0.05$.

Results

Cardiorespiratory parameters

The animals with an intra-arterial canula (IABP group) exhibited stable hemodynamic parameters throughout the experiments. Mean arterial pressure was within normal limits and remained above 65 mm Hg in all animals. Blood gas analysis showed normal pH, arterial carbon dioxide tension ($PaCO_2$), and arterial oxygen tension (PaO_2) levels with a small decrease in base excess (Table 1). Two of six animals in group R (recovery group) died directly after extubation. Before extubation, these animals did not differ from surviving subjects in cardiorespiratory parameters. The remaining four animals were stable during the ventilation-free interval, with normal activity and behavior and no respiratory distress or weight loss.

Histologic examination

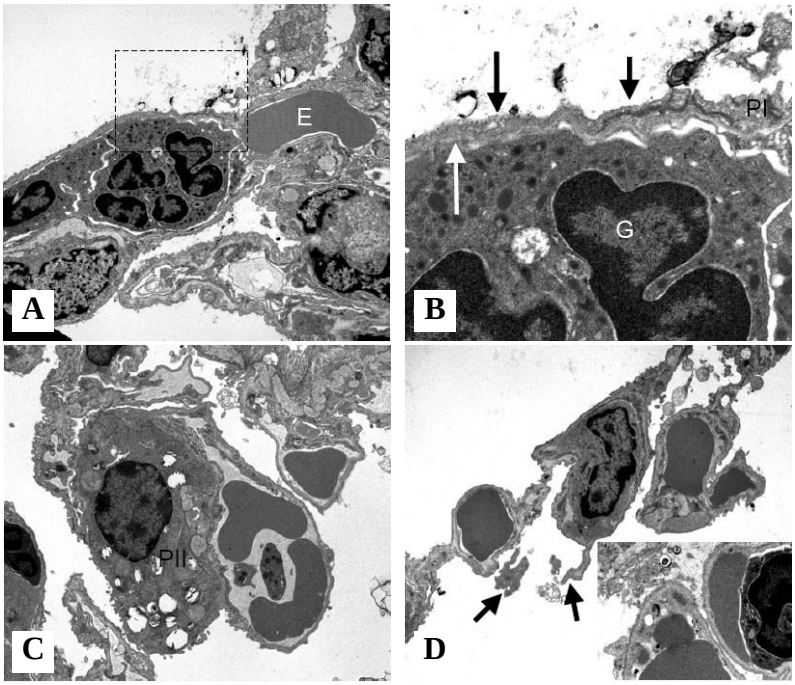
Electron microscopy examination of the lung tissue from animals in groups 30, 60, 120, and 240 revealed intact basement membranes and no signs of alveolar flooding. Type I pneumocytes sporadically showed signs of minimal membrane disruption and small partial detachment of endothelium (Figures 1A and B). Animals that were allowed to recover (group R) and unventilated animals (group C) showed no signs of membrane disruption or detachment of endothelium (Figure 1C). The four animals in the control experiment that were ventilated with a Vt of 16 ml/kg showed significant injury; lungs appeared overinflated (airtrapping) with loss of septal walls and injury of type I pneumocyte (Figure 1D). Light microscopy examination using periodic acid-Schiff staining showed no intra-alveolar albumin. Leder staining revealed a substantially

Table 1: *Intra-arterial blood pressure and arterial blood gas analysis during mechanical ventilation*

		Blood gas values				
Duration of MV min	**MAP mm Hg**	**pH**	**PaO_2 mm Hg**	**$PaCO_2$ mm Hg**	**HCO_3^-**	**BE**
0	102 (10)					
60	89 (12)					
120	83 (14)	7.36 (0.06)	229 (50)	38 (7)	20.4 (1.2)	-4.4 (1.5)
180	78 (6)					
240	79 (8)	7.35 (0.07)	194 (50)	36 (8)	19.6 (3.1)	-6.3 (2.0)

Values are mean (SD).
BE: base excess; MAP: mean arterial pressure; MV: mechanical ventilation; $PaCO_2$: arterial carbon dioxide tension; PaO_2: arterial oxygen tension.

Figure 1.



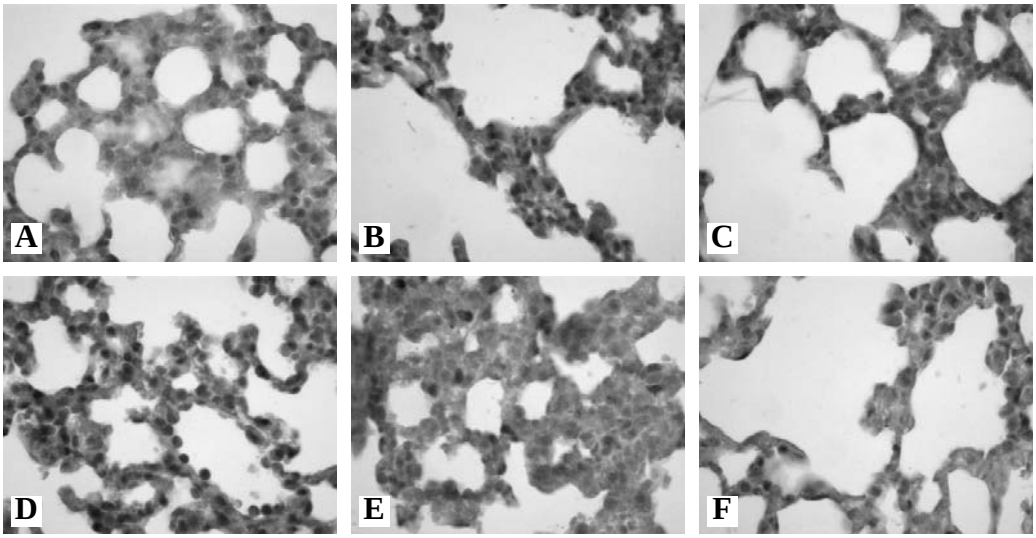
Electron microscopy. (A and B) Lung tissue of healthy animals after 240 min of mechanical ventilation. B is a magnification of a section of A (rectangle). Mostly the membrane of type I pneumocyte stayed intact (*black arrowhead, B*); sporadically, signs of membrane disruption and partial detachment of endothelium occurred (*black arrow, B*). Animals that were allowed to recover for 2 days (group R) and unventilated healthy control animals (group C) showed no signs of membrane disruption and no detachment of endothelium (C). Animals ventilated with tidal volume of 16 ml/kg showed extensive damage with lungs appearing overinflated with loss of septal walls (*black arrowheads, D*) and injury of type I pneumocyte and endothelium (inset, D). Magnification: A and C: 5000x; B: 15,000x; D: 3,000x (*inset* 7000x). E = erythrocyte; L = leukocyte; PI = type I pneumocyte; PII = type IIpneumocyte.

Table 2. *Leukocyte counts and wet / dry ratios*

Group	**Leukocytes x 10 ^{4}/ μm^{2} mean (SD)**	***p*-value**	**Wet / dry ratio mean (SD)**	***p*-value**
Control	2.1 (1.3)		4.68 (0.014)	
30	2.4 (1.6)	ns		
60	2.2 (2.2)	ns		
120	9.7 (5.0)	< 0.05	4.81 (0.13)	ns
240	5.7 (3.1)	< 0.05	5.01 (0.06)	< 0.05
Recovery	1.2 (0.9)	< 0.05*		

Values are mean (SD).
*p-values compared with control group (unventilated animals). * p-value compared with group 240 (240 min of ventilation), ns: not significant*

Figure 2.



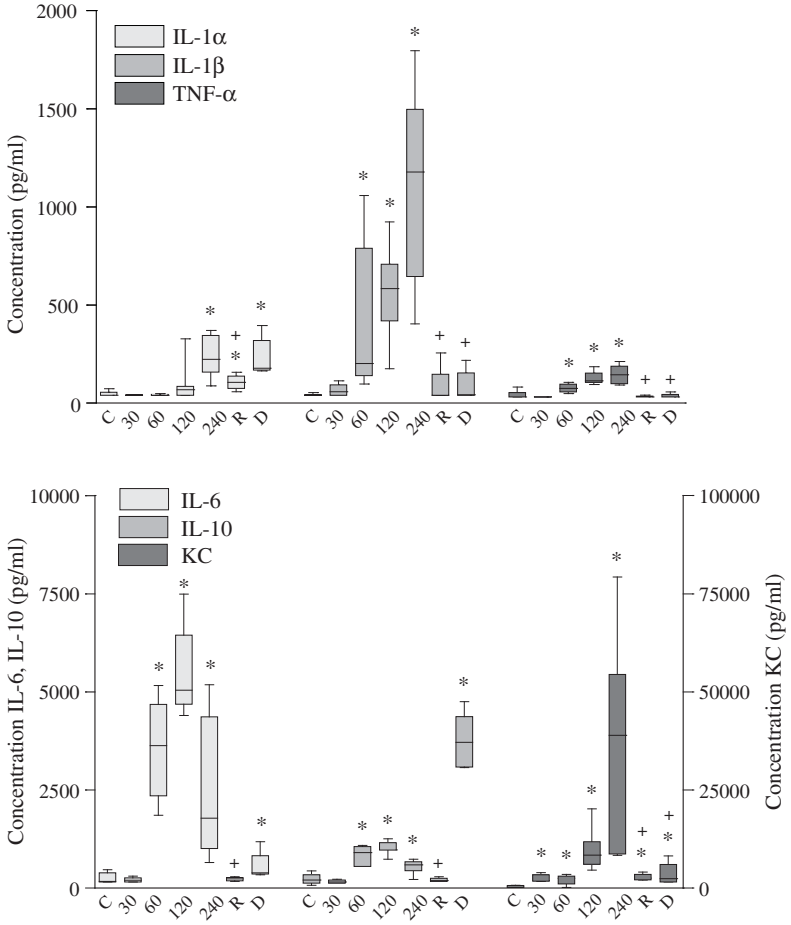
Light microscopy. Light microscopy examination of lung tissue after Leder staining revealed a significantly higher number of pulmonary leukocytes in healthy animals after 120 (group 120, *D*) and 240 min (group 240, *E*) of mechanical ventilation compared with the unventilated control animals (group *C, A*). Significantly lower numbers of pulmonary leukocytes were found in the animals that were allowed to recover (group *R, F*) compared with animals ventilated for 240 min (group 240, *E*). No differences were found between unventilated controls (group *C, A*) and the animals that were allowed to recover (group *R, F*). For the results of leukocyte counts, see Table 2. (*A*) Unventilated control animals. (*B-E*) Healthy animals receiving mechanical ventilation for 30, 60, 120, and 240 min. (*F*) Animals that were allowed to recover for 2 days after being ventilated for 240 min (group R). Magnification: 750x.

higher number of pulmonary leukocytes after 120 and 240 min of MV (Figure 2). No differences in leukocyte counts were found in the animals that were allowed to recover (group R) compared with the unventilated animals (group C). Wet/dry ratios showed increased ratios only after 240 min of MV. Data are presented in Table 2.

Cytokine concentration induced by mechanical ventilation

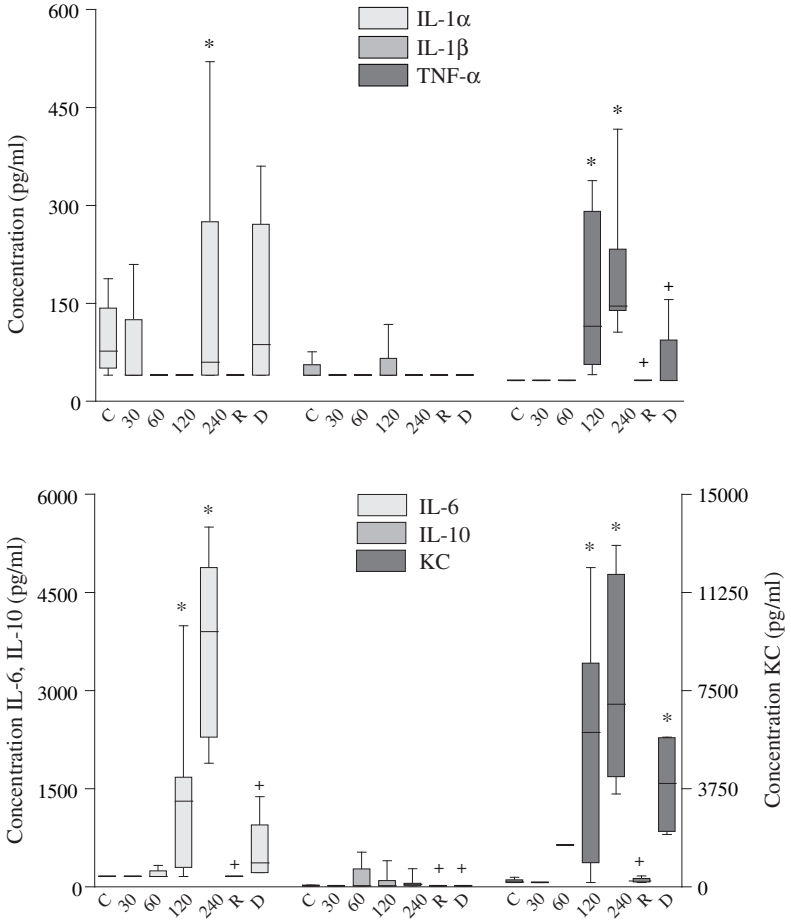
Mechanical ventilation with a Vt of 8ml/kg, PEEP of 4 cm H_2O, and FiO_2 of 0.4 resulted in a significant increase in IL-1α, IL-1β, TNF-α, IL-6, IL-10, and KC in lung tissue homogenate when compared with unventilated animals (group C). These cytokine concentrations increased with the duration of MV, with KC being the first to increase from 30 min of MV onward. IL-1β, TNF-α, IL-6, and IL-10 levels increased after 60 min, whereas IL-1α increased after 240 min compared with group C animals (Figure 3). In plasma, TNF-α, IL-6, and KC levels were elevated from 120 min onward compared with group C animals, and IL-1α after 240 min of MV. IL-1β and IL-10

Figure 3.



Cytokine levels in lung tissue homogenate. Mechanical ventilation significantly increased levels of interleukin (IL)-1α, IL-1β, tumor necrosis factor (TNF)-α, IL-6, IL-10, and keratinocyte-derived chemokine (KC) in lung tissue homogenate compared with unventilated animals (group C). When animals were allowed to recover for 2 days (group R), all lung tissue cytokine levels were found to be lower when compared with levels found in animals killed immediately after 240 min of ventilation (group 240). IL-1α and KC levels in group R animals were higher compared with group C animals. In leukocyte-depleted animals (group D), mechanical ventilation resulted in significantly higher levels of lung tissue IL-1α, IL-6, IL-10, and KC compared with group C animals. IL-1β, TNF-α, and KC levels were lower compared with the levels found after 240 min of mechanical ventilation in healthy animals (group 240). Data are expressed as box (median, 25th and 75th percentiles) and whiskers (range). * $p < 0.05$ compared with control. + $p < 0.05$ compared with group 240 (= 240 min of ventilation).- = Lower detection limit; 30, 60, 120, 240 = minutes of ventilation in healthy mice; C = control group (unventilated, healthy mice); D = leukocytedepleted group (these animals were leukocyte depleted before 240 min of mechanical ventilation and were killed immediately thereafter); R = recovery group (these animals were ventilated for 240 min and analyzed after 2 days of recovery).

Figure 4.



Cytokine levels in plasma. Mechanical ventilation significantly increases tumor necrosis factor (TNF)-α, interleukin (IL)-6, and keratinocyte-derived chemokine (KC) plasma levels from 120 min onward compared with unventilated animals (group C); IL-1α level was increased only after 240 min. When animals were allowed to recover for 2 days (group R) TNF-α, IL-6, IL-10, and KC were found to be lower when compared with levels found in animals killed immediately after 240 min of ventilation (group 240). In leukocyte-depleted animals (group D), mechanical ventilation resulted in significantly higher levels of plasma levels for KC compared with group C animals. TNF-α, IL-6, and IL-10 plasma levels were lower compared with the levels found after 240 min of mechanical ventilation in healthy animals (group 240). Data are expressed as box (median, 25th and 75th percentiles) and whiskers (range). * $p < 0.05$ compared with control. + $p < 0.05$ compared with group 240 (= 240 min of ventilation).- = Lower detection limit; 30, 60, 120, 240 = minutes of ventilation in healthy mice; C = control group (unventilated, healthy mice); D = leukocyte-depleted group (these animals were leukocyte depleted before 240 min of mechanical ventilation and killed immediately thereafter); R = recovery group (these animals were ventilated for 240 min and analyzed after 2 days of recovery).

levels were not different from those of the group C animals (Figure 4). When animals were allowed to recover for 2 days (group R), after being ventilated for 240 min, all lung tissue cytokine levels were lower compared with levels found in animals killed immediately after 240 min of ventilation (group 240). IL-1α and KC levels in group R animals were higher compared with group C animals. Plasma levels of TNF-α, IL-6, IL-10, and KC were found to be lower when compared with group 240.

The effect of leukocyte depletion on the release of cytokines

In leukocyte-depleted animals (group D), MV resulted in significantly higher levels of lung tissue IL-1α, IL-6, IL-10, and KC compared with group C animals. IL-1β, TNF-α, and KC levels were lower compared with the levels found after 240 min of MV in healthy (non-leukocyte-depleted) animals (group 240). MV resulted in higher plasma levels of KC compared with group C animals. TNF-α, IL-6, and IL-10 levels in plasma were lower compared with the levels found in group 240. No lipopolysaccharide could be detected in our experimental setting.

Discussion

The current study demonstrates that MV in healthy mice using clinically relevant ventilator settings with low Vt preserves alveolar integrity but induces reversible cytokine increase and leukocyte influx. This rapid increase in cytokine levels and leukocyte influx, however, does not result in persistent inflammation and VILI.

Our findings suggest that “noninjurious” or “lung-protective” ventilation does not exist and that even this careful mode of ventilation strategy leads to a reversible inflammatory response. Fortunately, MV in elective, healthy patients rarely leads to clinical significant injury. Apparently, in most circumstances, the lung is able to cope with the MV-induced inflammatory reaction. This is demonstrated in a clinical study by Plotz et al.,[36] which showed that 2 hours of MV (Vt 10 ml/kg) in healthy children, anesthetized for cardiac catheterization, resulted in elevated alveolar IL-6 and TNF-α concentrations without clinical signs of pulmonary dysfunction. In contrast with this are the findings in the clinical study of Wrigge et al.,[37] who found no ventilation-induced increase in cytokines. However, in this study, the ventilation duration was limited to 1 hour, and cytokines were only measured in plasma and not in the lung.

The current study is essentially different from previous experimental studies. In those studies, either injurious MV settings with large tidal volumes and high peak inspiratory pressures were used,[11,12,14,26,27,38] or lungs were preinjured using lipopolysaccharide, hydrochloric acid, or surfactant depletion.[13,15,16,28,29] Another major difference is that cytokine levels were measured in lung lavage fluid, whereas we used lung tissue

homogenate.[11,12,14,38] Lung lavage - by itself potentially injurious because it is often performed using large volumes [11,12,38] - requires cellular and alveolar leakage for the detection of cytokines. Analysis of lung tissue homogenate allows detection of cytokines before appearance in the alveolar space. This method of material harvesting does not allow the identification of the specific cells responsible for the measured increases in cytokine levels. However, the observed increase in KC after 30 min of MV, before the leukocyte influx, suggests a primary pulmonary origin of this cytokine. This is further supported by the finding of KC elevation in the leukocytedepleted animals (group D). However, we did not study the possible effects of cyclophosphamide on cytokine synthesis of pulmonary cells.

In this study, EM analysis revealed that our MV mode almost completely retained histologic integrity, with only sporadically minimal changes in a few samples. Most importantly, basement membranes were not disrupted, signifying alveolar integrity. Although LM has been used for quantitative analysis of leukocytes and can provide some qualitative evidence of lung injury (*i.e.*, alveolar flooding, thickening of alveolar septa),[39,40] more detailed evaluation of structural changes in the lung requires EM.[41] The effects of large Vt in the animals ventilated with Vt of 16 ml/kg was clearly visible with EM as damage with loss of compartmentalization, consistent with findings in a previous publication.[9] We observed increased wet / dry ratios in animals after 240 min of MV. However, we conclude that this increased wet / dry ratio (without any other signs of possible lung damage as shown by EM and LM examination) is not of clinical significance. This is supported by the finding of the complete recovery of the animals who were allowed to recover after 240 min of ventilation (group R).

Factors affecting cytokine response other than MV were carefully avoided. The possibility of triggering an inflammatory response by invasive procedures, (*i.e.*, insertion of an intra-arterial line)[18] was eliminated by performing our experiments in noninvasively monitored animals, after having documented cardiorespiratory stability in invasively monitored animals (IABP group). A limulus amebocyte lysate test excluded possible aerogenic lipopolysaccharide contamination during our experiments. Cardiorespiratory parameters and the choice of the anesthetics are known to influence the cytokine profile. In the current study, mean arterial pressure was maintained above 65 mm Hg, and blood gas analysis showed normal pH, $PaCO_2$, and PaO_2 levels. Only a small decrease in base excess after 120 and 240 min of MV was observed, comparable with other studies.[11,12] The slight decrease in base excess in the presence of a normal mean arterial pressure unlikely interferes with our observations. The effect of anesthetics on hemodynamic stability in mice has been studied extensively by Zuurbier et al.,[42] who found KMA mix superior compared with other regimens (e.g., fentanyl-fluanisone-midazolam mix or isoflurane). Some anesthetics, e.g., propofol,[43] volatile

anesthetics,[44,45] and ketamine,[46,47] are known to influence cytokine profiles. Ketamine is known to have an inhibitory effect on lipopolysaccharide-induced cytokine production.[46,48-50] In the current study, all animals received the KMA mix. Ideally, an additional control group of spontaneously breathing animals under KMA anesthesia is needed. However, this will result in hypoventilation with severe respiratory acidosis and hemodynamic instability. Two mice in group R (recovery group) died immediately after extubation; the cause of death was due to airway problems related to residual effects of the anesthesia. The other mice in group R made uneventful recoveries. By excluding these confounding factors, we attribute the increase in cytokine levels to MV.

Therefore, even low-Vt MV induces an inflammatory response and, in a "multiple-hit" situation, might be the additional "proinflammatory hit" resulting in lung injury. Modulation of the inflammatory response may offer strategies to reduce VILI. In this respect, anesthetics may play a role because volatile anesthetics have been shown to exhibit antiinflammatory effects in different organ systems and might be able to modulate the release of cytokines.[51] The influence of different anesthetics on the inflammatory response in our model needs further investigation. Recently, Jiang et al.[52] discovered that Toll-like receptor 4-mediated inflammation by endogenous compounds might also be important in the development of VILI. Further study of the Toll-like receptors and the molecules with which they interact may reveal more insight into the molecular mechanisms of VILI.

Conclusion

The current study shows that in healthy male mice, a short period of "noninjurious" ventilation induces a reversible inflammatory reaction, while preserving tissue integrity. This model offers opportunities to study the pathophysiologic mechanisms of VILI and the contribution of MV to the "multiple-hit" concept.

Abbreviations

MV	mechanical ventilation
VILI	ventilator-induced lung injury
Vt	tidal volumes
PEEP	positive end-expiratory pressure
KMA	combination of ketamine, medetomidine, and atropine
IABP	intra-arterial blood pressure
LM	light microscopy
EM	electron microscopy

IL	interleukin
TNF	tumor necrosis factor
KC	keratinocyte-derived chemokine

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Chapter 4

Hypercapnic acidosis attenuates the pulmonary innate immune response in ventilated healthy mice

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Abstract

Background: Mechanical ventilation with small tidal volumes reduces the development of ventilator induced lung injury and mortality, but may increase $PaCO_2$. It is not clear whether the beneficial effect of a lung-protective strategy results from reduced ventilation pressures / tidal volumes or is mediated by the effects of hypercapnic acidosis on the inflammatory response involved in the pathogenesis of ventilator induced lung injury.

Objective: To analyze whether hypercapnic acidosis affects lung tissue cytokine levels and leukocyte influx in healthy ventilated mice.

Study Design: Analysis of lung tissue and plasma concentrations of IL-1β, TNF-α, IL-6, IL-10, and keratocyte-derived chemokine after 2 hours of mechanical ventilation (Vt 8 ml/kg, PEEP 4 cm H_2O) with 0.06% CO_2 (room air), 2% CO_2, or 4% CO_2.

Subjects: Healthy C57BL6 mice (n = 40).

Measurements / Results: $PaCO_2$ and pH were within normal range when ventilated with 0.06% CO_2 and significantly changed with 2% and 4% CO_2: (mean ± SD) pH 7.23 ± 0.06 and 7.15 ± 0.04, $PaCO_2$ 7.9 ± 1.4 and 10.8 ± 0.7 kPa, respectively ($p < 0.005$). Blood pressure remained within normal limits in all animals. Quantitative microscopic analysis showed a 4.7 ± 3.7-fold increase ($p < 0.01$) in pulmonary leukocyte influx in normocapnic ventilated animals and a significant reduction in leukocyte influx of 57 ± 32% ($p < 0.01$) and 67 ± 22% ($p < 0.01$) when ventilated with 2% and 4% CO_2, respectively. Normocapnic ventilation induced a significant elevation of lung tissue IL-1β (1516 ± 119 ng/ml), TNF-α (344 ± 88 ng/ml), IL-6 (6310 ± 807 ng/ml), IL-10 (995 ± 152 ng/ml), and keratocyte-derived chemokine (36966 ± 15294 ng/ml) (all *p*-values < 0.01). Hypercapnic acidosis with 2% respectively 4% CO_2 significantly attenuated this increase with 25 ± 32% and 54 ± 32% (IL-1β, $p < 0.01$); 17 ± 36% and 58 ± 33% (TNF-α, $p < 0.02$); 22 ± 34% and 89 ± 6% (IL-6, $p < 0.01$); 20 ± 31% and 67 ± 17% (IL-10, $p < 0.01$) and 16 ± 44% and 45 ± 30% (keratocyte-derived chemokine, $p = 0.07$).

Conclusion: Hypercapnic acidosis attenuates the mechanical ventilation-induced immune response independent from reduced tidal volumes / pressures and may protect the lung from ventilator induced lung injury.

Introduction

Mechanical ventilation (MV) can induce or exacerbate lung injury through a process of baro-volutrauma and inflammation, termed *ventilator induced lung injury* (VILI). Reducing tidal volumes and peak pressures ("Lung Protective Ventilation") not only results in both lower pulmonary cytokine levels but also decreased lung injury and mortality.[1-3] However, low tidal volume ventilation may result in an increase in $PaCO_2$ ("permissive hypercapnia"). Severe hypercapnic acidosis is usually well tolerated [4, 5] and several clinical studies even suggest a protective effect on various organs, including the ventilated lung.[6-9] A retrospective multivariate logistic regression analysis of the ARDS network trial [10] suggests that hypercapnic acidosis may be protective in ARDS patients ventilated with high tidal volumes.

Several mechanisms may explain the effect of hypercapnic acidosis on VILI. First, accepting a higher $PaCO_2$ level allows the use of smaller tidal volumes and lower airway pressures, which has beneficial effects on VILI, as shown in several clinical trials.[5-7]. Second, hypercapnic acidosis has direct effects on lung perfusion, oxygenation and oxygen delivery. Both experimental and clinical studies have shown that hypercapnic acidosis increases tissue oxygen delivery, by shifting the oxygen dissociation curve to the right and improving V / Q match.[11-13] Third, an increase in $PaCO_2$ may have a direct modulating effect on the inflammatory response. Some of these effects are probably protective, such as inhibition of xanthine oxidase and oxygen radical formation,[14] decreased NF-kB transcriptional activity[15] and decreased complement activation.[16] Other effects are potentially harmful, e.g., reduced neutrophil burst and superoxide formation,[16] inhibition of NO synthases [17] and delayed apoptosis. [18,19] The biochemical pathways on a cellular level include altered electrochemical membrane potentials, alterations in microtubuli assembly, lowered enzyme activity and altered gene transcription as reviewed by Kregenow et al.[16]

In the clinical setting it is difficult to establish which of the two variables, reduced tidal volume / pressure or hypercapnic acidosis, accounts for the observed improvement in outcome. We conducted a series of experiments to establish the effect of hypercapnic acidosis on ventilationinduced pulmonary cytokine increase and leukocyte influx by administration of inhaled CO_2, while maintaining identical ventilator settings. We show that hypercapnic acidosis significantly decreases lung leukocyte influx and cytokine release.

Materials and methods

All experiments were approved by the Regional Animal Ethics Committee Nijmegen and performed under the guidelines of the Dutch Council for Animal Care and the National Institutes of Health.

Animals

C57BL6 mice (n = 40, Charles River, Sulzfeld, Germany), 10-12 wks of age, with a weight ranging from 23 to 28 g were used.

Mechanical ventilation

Anesthesia was induced and maintained with intraperitoneal administration of a combination of ketamine, medetomidine, and atropine as reported before.[20] Animals were orally intubated under direct vision with an endotracheal tube (0.82 mm ID, 1.1 mm OD, length 25 mm). Subsequently, animals were connected to the ventilator (MiniVent®, Hugo Sachs Elektronik - Harvard apparatus, March-Hugstetten Germany). Tidal volume (Vt) was set at 8 ml/kg, which is comparable with Vt during spontaneous ventilation in C57BL6 mice.[21] All animals were ventilated with 4 cm H_2O PEEP. Studies using bronchoalveolar lavage did not find increased cytokine levels with similar ventilatory settings; however, using tissue homogenate we previously demonstrated increased intracellular and tissue cytokine levels, without evoking histologic changes.[20,22-24]

To avoid direct oxygen toxicity [25,26] the FiO_2 was set at 0.4. Respiratory rate was set and fixed at 150/min to obtain normocapnia with 0.06% CO_2 (room air). An intra-arterial carotid cannula was inserted for continuous blood pressure monitoring and blood gas analysis at the end of the experiment. Throughout the experiment rectal temperature was monitored and maintained between 36.5° and 37.5° C using a heating pad.

Study groups

In the first set of experiments, animals were randomly divided into 4 groups. Group I (n = 6) was not ventilated, immediately killed after induction of anesthesia and served as the control group. Group II (n = 6) was ventilated with 0.06% CO_2. Group III (n = 6) was ventilated with 2% CO_2, and group IV (n = 6) was ventilated with 4% CO_2. Ventilator settings in group II, III, and IV were identical as described above.

In a second set of experiments we used the same protocol to measure pulmonary wet / dry ratios. These animals were ventilated as in group II (n = 4), III (n = 4), and IV (n = 4). Four spontaneously breathing animals served as controls (n = 4).

Material harvesting and preparation of lung tissue

Mechanical ventilation lasted 2 hrs in all groups, after which the mice were killed by exsanguination. Blood was collected from the arterial line, centrifuged at 13000 g (Eppendorf 5415 C, Nethler- Hinz GmbH, Hamburg) for 2 mins and the plasma was stored at -80° C. Immediately after exsanguination, the heart and lungs were carefully removed *en bloc* via midline sternotomy. The right middle lobe was fixed for light microscopy. The remaining lung tissue was homogenized for the determination of cytokine concentrations.

To assess pulmonary edema, wet / dry ratios of both lungs were used; ratios were calculated by measuring lung weight before and after heating for 24 hours in a stove at 40° C.

Light microscopy

For light microscopy the material was fixed in 4% buffered formalin solution overnight at room temperature, dehydrated and embedded in paraplast (Amstelstad, Amsterdam, The Netherlands). Sections of 4 μm-thickness were used for analysis. The enzyme activity of leukocytes was visualized by enzyme histochemistry using chloracetatesterase staining (Leder staining). Leukocytes were counted manually (20 fields per mouse), and after automated correction for air / tissue ratio, leukocytes/mm^2 were calculated. The pathologist was blinded for the group and ventilation protocol.

Laboratory tests

Levels of TNF-α, IL-6, IL-10, and keratocyte-derived chemokine (KC) in lung homogenate were measured using enzyme-linked-immunosorbent assay (ELISA) (for TNF-α, IL-6 and IL10; CytoSet, BioSource, USA; for KC; ELISA-Kit, R&D, USA). IL-1β was assessed using specific radioimmuno-assays (Nijmegen University, Netherlands), as described previously.[20,27]. Lower limit of detection for IL-1β was 40 pg/ml, for TNF-α 32 pg/ml, for IL-6 160 pg/ml, for IL-10 16 pg/ml, and for KC 160 pg/ml.

Statistical analysis

Data are expressed as mean ± SD. If cytokine concentrations were below the detection limit, lower limits of detection were used for further calculations. Comparison between groups was performed using one-way ANOVA. A p-value < 0.05 was considered statistically significant. All tests and graphs were constructed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

Table 1. *Arterial blood gas analysis*

	pH	PaO_2 kPa	$PaCO_2$ kPa	HCO_3^-	BE
0.06% CO_2	7.36 ± 0.06	28.9 ± 10.4	5.0 ± 0.9	20.5 ± 1.2	-4.4 ± 1.5
2% CO_2	7.23 ± 0.06*	27.2 ± 4.9	7.9 ± 1.4*	21.4 ± 0.9	-4.5 ± 1.2
4% CO_2	7.15 ± 0.04*	28.9 ± 1.3	10.8 ± 0.7*	22.7 ± 1.8	-3.8 ± 2.8

*$p < 0.005$ 2%, 4% CO_2 vs. 0.06% CO_2.

Table 2. *Arterial blood pressure (mm Hg)*

	***T* = 0.5 hour**	***T* = 1 hour**	***T* = 1.5 hour**	***T* = 2 hour**
0.06% CO_2	95.6 ± 15.7	95.3 ± 15.9	78.6 ± 8.1	80.1 ± 10.2
2% CO_2	92.1 ± 14.3	78.6 ± 6.9	76.2 ± 7.9	74.3 ± 2.4
4% CO_2	92.5 ± 5.1	85 ± 7.2	79.25 ± 3.3	81.5 ± 8.1

Results

Cardio respiratory parameters

As illustrated in Table 1, animals ventilated without supplemental CO_2 (group II) exhibited normal pH, PaO_2 and $PaCO_2$ at the end of experiment, comparable with values in spontaneously breathing mice. Increasing inspiratory CO_2 (group III and group IV) resulted in a significantly elevated $PACO_2$ and a decrease in pH ($p < 0.05$) (Table 1), whereas base excess did not differ between groups. In all animals, invasive arterial blood pressure remained within normal limits throughout the experiment and did not differ between groups (Table 2).

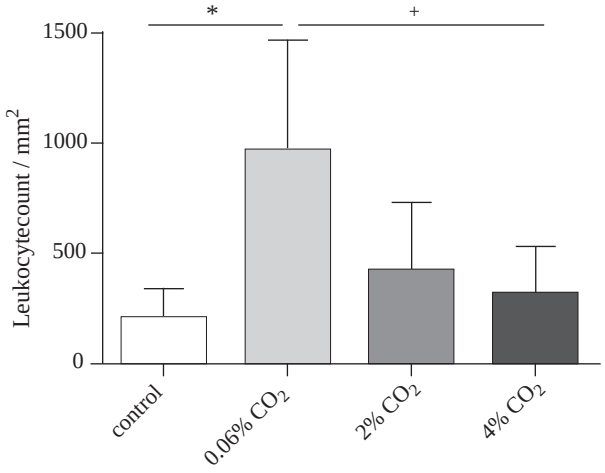
Light microscopy

Mechanical ventilation significantly increased lung leukocyte counts in animals ventilated with 0.06% CO_2 from 207 ± 133 x mm^2 to 971 ± 496 mm^2 (group I vs. group II, $p < 0.01$). MV with 2% and 4% CO_2 (group III and IV) attenuated the increase in leukocyte influx with 57 ± 32% and 67 ± 22% ($p < 0.01$), respectively (Figure 1). No alveolar tissue disruption or other structural damage was observed and all detected leukocytes remained within the lung interstitium.

Wet / dry ratio

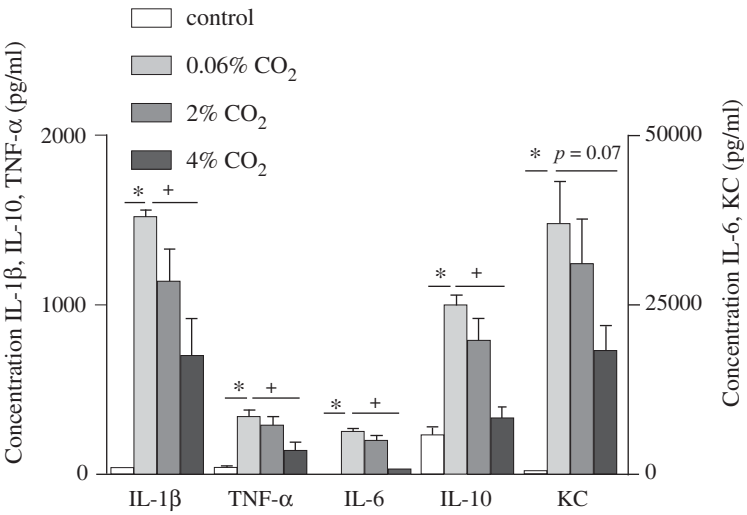
There were no significant differences in wet / dry ratios between the four groups (data not shown, all $p > 0.2$).

Figure 1.



Leukocyte influx. Effect of 2 hours normo- and hypercapnic mechanical ventilation on leukocyte counts in lung tissue, compared with unventilated control animals. * $p < 0.05$; + $p < 0.05$: 0.06% CO_2 vs. 2% CO_2 vs. 4% CO_2.

Figure 2.



Lung tissue cytokines. Effect of 2 hours normo- and hypercapnic mechanical ventilation on ventilation-induced lung tissue cytokine release using identical ventilator settings. * $p < 0.05$ vs. control; + $p < 0.05$: 0.06% CO_2 vs. 2% CO_2 vs. 4% CO_2.

Cytokine concentrations

IL-1β, TNF-α, IL-6, IL-10, and KC lung tissue levels were significantly increased after 2 hours of normocapnic MV: IL-1β from 42 ± 4 to 1516 ± 119 pg/ml; TNF-α from 42 ± 18 to 344 ± 88 pg/ml; IL-6 from 264 ± 130 to 6310 ± 807 pg/ml; IL-10 from 233 ± 124 to 995 ± 152 pg/ml; KC from 384 ± 217 to 36967 ± 15294 pg/ml (all *p*-values < 0.01). In group III (2% CO_2) and group IV (4% CO_2), hypercapnic MV significantly attenuated this increase in IL-1β with 25 ± 32% and 54 ± 32% ($p = 0.006$); TNF-α 17 ± 36% and 58 ± 33% ($p = 0.019$); IL-6 22 ± 34% and 89 ± 6% ($p = 0.001$); IL-10 20 ± 31% and 67 ± 17% ($p = 0.005$), and KC 16 ± 44% and 45 ± 30% ($p = 0.07$) (Figure 2).

Discussion

In the present study, we demonstrate that the ventilation-induced increase in leukocyte influx and pulmonary cytokines, a hallmark of VILI, is significantly attenuated in hypercapnic-acidotic animals ventilated with the same tidal volumes. Hypercapnic acidosis may represent an important pathway to protect the lung against VILI. To our knowledge, this study demonstrates for the first time the attenuating effect of hypercapnic acidosis on cytokine levels *in-vivo*. As all other cardiorespiratory and ventilatory parameters remained identical between ventilated groups, we assume that the observed differences are entirely attributable to differences in hypercapnic acidosis.

Our *in-vivo* findings are in concordance with several *in-vitro* observations. Lang et al.[28] showed that hypercapnia attenuates TNF-α production in lipopolysaccharide-stimulated rabbit and rat alveolar macrophages *in-vitro*. In human, lipopolysaccharide-stimulated polymorphonuclear neutrophils and alveolar macrophages hypercapnic acidosis decreased the production of IL-8.[15,29] IL-8 is a potent chemoattractant and comparable with KC in animals. We showed an attenuated increase in KC during ventilation with hypercapnic acidosis. We previously demonstrated that mice ventilated for 30 mins with identical ventilator settings had increased KC levels in lung homogenate before leukocyte influx, and that leukocyte depleted mice demonstrated a significant mechanical ventilation-induced increase in KC but hardly any increase in IL-1β, TNF-α, IL-6, and IL-10.[20] The attenuated KC increase during hypercapnic acidosis may explain the decrease in leukocyte infiltration as observed by us and by others studying nonventilation- related primary lung injury (pulmonary lipopolysaccharide installation) and secondary lung injury (ischemia reperfusion) *in-vivo*.[30-32]

Once cytokine up-regulation and leukocyte chemotaxis are triggered by mechanical ventilation, further propagation of the inflammatory response by release of cytokines

from leukocytes through positive feedback occurs. Experimental administration of a IL-1β receptor antagonist and TNF-α monoclonal antibodies appear to attenuate the development of VILI, demonstrating the involvement of cytokines in its pathogenesis.[33-35] Moreover, several clinical studies clearly demonstrated the relation between cytokine levels and morbidity.[36,37] Another cytokine-propagating mechanism affected by hypercapnic acidosis may be decreased NF-κB activation. NF-κB appears to be stimulated by both IL-1β and TNF-α and regulates gene transcription responsible for IL-6 and KC synthesis.[38] Hypercapnic acidosis inhibits NF-κB translocation into the nucleus by suppressing degradation of IκB-α, a regulatory protein that binds the cytoplasmatic inactive NF-κB.[15,39] This pathway may offer an explanation for the reported attenuation of NF-κB mediated IL-6 and KC increase in hypercapnic acidosis. Further experiments are necessary to elucidate what the effects of hypercapnic acidosis during MV are on the cellular mechanisms proposed above.

From our experiment, it remains unclear whether it is the hypercapnic acidosis or the resultant acidosis that is responsible for the observed beneficial effects. In *ex-vivo* lung injury experiments, hypercapnic acidosis appears to preserve alveolocapillary integrity more than metabolic acidosis.[40] Also, synergistic effects between $PaCO_2$ and pH may occur, as buffer therapy for hypercapnic acidosis reduces its protective effects.[40] A limitation of our study model is that the ventilator setting, although clinically relevant, results in functional dysregulation with elevated cellular and tissue cytokine levels, but not in histologic lung injury.[20] Therefore, our study does not enable us to conclude that hypercapnic acidosis also better preserves pulmonary integrity. However, the conclusions regarding the pathophysiological importance of the increased cytokine levels still hold. As ventilation duration in our study was limited to 2 hours, the observed beneficial effects cannot directly be extrapolated to outcome when ventilated for longer periods. Also, whether hypercapnic acidosis also attenuates the cytokine response in an injured lung with an otherwise activated innate immune response as encountered in the clinical setting, needs further study.

Conclusion

Hypercapnic acidosis by itself directly attenuates pulmonary leukocyte influx and cytokine release during mechanical ventilation, an effect that is independent from reduced tidal volume and pulmonary pressures. The observation that hypercapnic acidosis directly modulates the mechanical ventilation-induced innate immune response in the lung suggests that this pathway may account for the beneficial effects of hypercapnic acidosis in critically ill ventilated patients. The net effect of hypercapnic acidosis on pulmonary and other end organ function in the clinical setting remains to be determined.

Abbrevations

MV	mechanical ventilation
VILI	ventilator-induced lung injury
IL	interleukin
TNF	tumor necrosis factor
KC	keratinocyte-derived chemokine
PEEP	positive end-expiratory pressure
Vt	tidal volume
NF-κB	nuclear factor κB

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Section II

Clinical studies in Children in the ICU

Chapter 5

Lung recruitment during mechanical positive pressure ventilation in the Pediatric Intensive Care Unit: *what can be learned from the literature?*

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Abstract

A literature review was conducted to assess the evidence for recruitment maneuvers used in conventional mechanical positive pressure ventilation. A total of 61 studies on recruitment maneuvers were identified: 13 experimental, 32 ICU, 7 PICU and 12 anaesthesia studies. Recruitment appears to be a continuous process during inspiration and expiration and is determined by peak inspiratory pressure (PIP) and positive end expiratory pressure (PEEP). Single or repeated recruitment maneuvers may result in a statistically significant increase in oxygenation; however, this is short lasting and clinically irrelevant, especially in late ARDS and pneumonia. Temporary PIP elevation may be effective but only after PEEP loss (for example disconnection and tracheal suctioning). Continuous PEEP elevation and prone positioning can increase PaO_2 significantly. Adverse haemodynamic or barotrauma effects are reported in various studies. No data exist on the effect of recruitment maneuvers on mortality, morbidity, length of stay or duration of mechanical ventilation. Although recruitment maneuvers can improve oxygenation, they can potentially increase lung injury, which eventually determines outcome. Based on the presently available literature, prone position and sufficient PEEP as part of a lung protective ventilation strategy seem to be the safest and most effective recruitment maneuvers. As paediatric physiology is essentially different from adult, paediatric studies are needed to determine the role of recruitment maneuvers in the PICU.

Introduction

In recent years it has become evident that mechanical ventilation can cause and perpetuate lung disease: this is known as ventilator induced lung injury (VILI).[1-5] Attention has been directed towards lung protective ventilation strategies (LPVS) resulting in the ARDS network trial, which showed that the use of tidal volumes of 6 ml/kg significantly reduced mortality compared with volumes of 12 ml/kg.[6] However, low tidal volume ventilation results in lung de-recruitment and lung consolidation / atelectasis and can result in increased intrapulmonary shunting. Hence various recruitment strategies and maneuvers, single or repeated, are superimposed on LPVS to increase alveolar recruitment and improve oxygenation. Although widely used in clinical practice, their beneficial effects have never been proven, and evidence exists that recruitment maneuvers might even be harmful.[7-12] As lung recruitment and derecruitment is a continuous process during tidal ventilation, it is likely that the effect of recruitment maneuvers depends on both the ventilator settings and the particular pulmonary condition. Recruitment maneuvers should therefore be adjusted to the individual patient. Although physiology and pathophysiology in the Paediatric Intensive Care Unit (PICU) may be essentially different from that in the adult ICU, evidence based guidelines or double-blind randomised controlled trials are often lacking and paediatric intensivists have to rely on studies performed in the adult ICU. This paper reviews the existing literature on recruitment maneuvers during positive pressure ventilation in experimental, ICU, anaesthesia and PICU departments with special emphasis on implications for the PICU.

Experimental studies on alveolar recruitment (Table 1).

Direct microscopy has shown that the healthy lung is recruited during the entire ventilatory cycle, and that only 20% of the volume increase is a result of alveolar distention, whereas the remaining 80% is due to alveolar opening and closure.[13] The driving force for alveolar opening during inspiration is the peak inspiratory pressure (PIP) (Figure 1).[14,15] During mechanical ventilation, alveoli have a tendency to collapse during expiration due to their elasticity and gravitational forces, and PEEP appears to maintain alveolar patency and prevent de-recruitment (Figure 2).[14-17]

In the diseased lung, direct microscopy shows a mixture of alveoli behaving normally, alveoli showing extreme over-distention during inspiration and alveoli collapsing during expiration.[18] It is likely that these latter alveoli are prone to shear stress induced injury. Higher inspiratory and expiratory pressures are needed to open and stabilise these collapsed alveoli, and the increased inhomogeneity is reflected in different opening, over-distension and collapse pressures within the lung.[19-21] The

Table 1. *Experimental studies on lung recruitment*

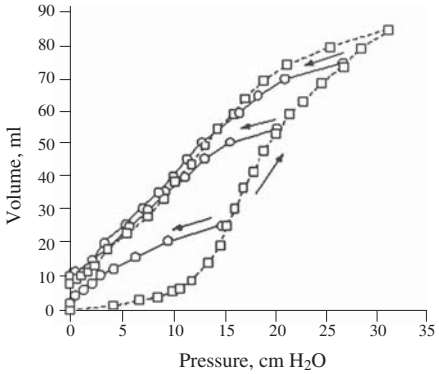
Author	Lung status	Recruitment Mode	Study design	Outcome: Oxygenation	Outcome: resp mechanics	Outcome: VILI effects
Suh [43]	ALI (lavage)	Cont. PEEP	A: PEEP 12 vs B: PEEP 2 vs C: PEEP 12-2-12-2-12	A: PaO_2 66 B: PaO_2 5.3 C: PaO_2 29		Most hyaline membranes in last group
Muscedere [40]	ALI (lavage)	Cont. PEEP	A: PEEP > LIP vs B: PEEP < LIP		A: Increased compliance	A: Less pulmonary injury
Luecke [108]	ALI (lavage)	Cont. PEEP	PEEP 0-7-14-21 with PIP35 vs 45	PaO_2 increases with PEEP	PEEP increase neutralises PIP reduction	
Kloot [31]	ALI	Sustained PEEP	RM: sust PEEP 60 in: lavage model/ oleic acid model/ pneumonia	 + – –	 – + –	
Rimensberger [14]	ALI (lavage)	Sust. PEEP	A: RM: sust PEEP 30 B: no RM	A: PaO_2 57 B:18 effect > 4 hours	A: EELV +	
Rimensberger [109]	ALI (lavage)	Sust PEEP	A: PEEP < LIP B: PEEP < LIP + RM C: PEEP > LIP + RM RM: Sust PEEP 30	C: best compliance		B: least VILI
Fujino [30]	ALI (lavage)	PEEP/ PIP	A: Sust PEEP 40 vs B: Sust PEEP/PIP 40/60	A: PaO_2 13 B: PaO_2 50		A = B
Bjorklund [38]	IRDS in prematurity	PIP	6 inflations 40 ml/kg at birth		Worsened	Worsened

Table 1. *(Continued)*

Author	Lung status	Recruitment Mode	Study design	Outcome: Oxygenation	Outcome: resp mechanics	Outcome: VILI effects
Carney [13]	healthy		Direct alveolar microscopy		Lung volume increase during ventilatory cycle: 20% alveolar distention 80% alveolar recruitment	
Halter [20]	ALI (lavage)	PEEP/PIP	Direct microscopy RM: PIP 45 at A: PEEP 5 B: PEEP 10	baseline PaO_2 7.5 A: PaO_2 22 B: PaO_2 46	PIP recruits alveoli, PEEP10 stabilises more than PEEP5	
McCann[19]	ALI (surfactant inactivation)	PEEP	Direct microscopy ZEEP vs PEEP		Diminished alveolar instability with PEEP	
Lamm [33]	ALI (oleic acid)	Prone	A: Prone B: Supine	A: PaO_2 18 B: PaO_2 46	V/P in ALI improves	
Lim [32]	ALI (lavage)	Prone	Prone pos. vs supine at different PEEP levels	PaO_2 Prone > supine increase from low PEEP > high PEEP		

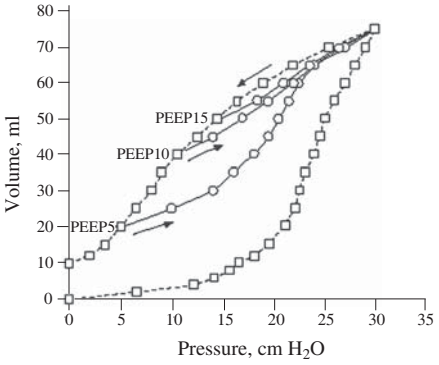
RM: recruitment maneuver; PEEP: positive end expiratory pressure; ZEEP: zero end-expiratory pressure; PIP: peak inspiratory pressure, pressures in cm H_2O; LIP: lower inflection point; PC: Pressure control; sat: oxygen saturation in percentage; PaO_2 in kPa; PP: prone position; sust: sustained. cont: continuous; EELV: end-expiratory lung volume; ALI: acute lung injury; VILI: ventilator-induced lung injury; V/P: ventilati on/perfusion ratio.

Figure 1.



Rimensberger et al. inflation of saline-lavaged rabbit lungs exhibit the same inflation curves, but greater volumes at equal pressures after inflation to higher PIP, indicating more recruitment.[14]

Figure 2.



Rimensberger et al. deflation PV-curves of saline-lavaged rabbit lungs exhibit the same inflation curves and less hysteresis when deflated to higher PEEP levels, indicating less de-recruitment with higher PEEP levels.[14]

upper inflection point on the pressure-volume loop is related to alveolar over-distention, usually at pressures well over 30 - 50 cm H_2O.[22,23] The lower inflection point (LIP) is not related to alveolar opening or optimal recruitment or 'optimal PEEP' as it is merely a result of external factors such as chest wall compliance and intra-abdominal pressure, and the recruitment state as a result of volume history.[22,24-28] Besides direct microscopy, studies analysing pressure / volume (PV)-loops show that alveolar recruitment occurs above LIP until the UIP.[17,29] With the use of the PV-loop the role of PIP to recruit and PEEP to limit de-recruitment during expiration has been confirmed.[14,15,17]

Only one of the experimental studies on recruitment maneuvers showed a prolonged effect on oxygenation improvement lasting for 4 hours after a single sustained PEEP recruitment maneuver; however, 65% of the animals developed pneumothoraces and showed a significant decrease in cardiac output.[14] In another study by the same authors, sustained PEEP recruitment maneuvers in LPVS diminished histological lung injury, a finding that was not reported by others.[30] In the other recruitment maneuver studies that show improvement of oxygenation, the effect is usually short lasting.[30,31] Only studies using prone positioning as a recruitment procedure show a longer effect on oxygenation, an effect that mostly disappears after repositioning to the supine position.[32,33]

Recruitment maneuvers using high pressures however, may result in barotraumas or volutraumas or ventilator induced lung injury. Verbrugge et al. observed translocation of bacteria after recruitment maneuvers using high pressure (> 45 cm H_2O).[34]. Lim et al. found similar results when using larger compared with smaller tidal volumes.[35]

A subsequent study by Cakar could not confirm this finding.[36] However, even a seemingly harmless recruitment maneuver such as ballooning is known to result often in very high pressures (> 60 cm H_2O).[37] The clinical relevance of this is demonstrated by a study of Bjorklund in which six manual inflations of 35-40 ml/kg applied to premature lambs immediately after birth resulted in more extensive histological lung injury and worsened lung mechanics when compared with lambs directly ventilated with LPVS.[38] Interestingly, Kloot et al. found an improvement in oxygenation after a recruitment maneuver using PEEP only in a lung lavage model, but not in an oleic acid and pneumonia model; an increase in PEEP level was only effective when low values of PEEP and small tidal volumes were used.[31]

The importance of sufficient PEEP level in acute lung injury and ARDS to reduce ventilator induced lung injury has been studied extensively and is widely accepted as one of the most important factors attenuating VILI, although increasing PEEP to very high values also results eventually in lung injury.[4,6,19,39-42] Repeated de-recruitments due to intermittent loss of PEEP are also known to be injurious.[43] Therefore a ventilation strategy with sufficient PEEP and limited PIP seems to be more important than single recruitment maneuvers for both short-term effects such as adequate oxygenation, and long-term effects such as limitation of VILI and possibly ventilator associated pneumonia (VAP).

Human studies

Anaesthesia and ICU (Table 2)

Studies on recruitment maneuvers have been the subject of discussion since they were first published.[7-9,12,44-47] As in the experimental setting, recruitment and de-recruitment in humans also occurs through the entire ventilatory cycle, the extent depending on the relationship between the pulmonary condition and the ventilator setting.[11,17,26,29,48] Lung consolidation in the ICU is not only caused by high FiO_2 and gravitational forces as during anaesthesia, but also by surfactant dysfunction and alveolar flooding due to an altered vascular barrier.[49,50] The pressure needed to open consolidated lung areas in late ARDS or pneumonia appears to be higher (> 45 cm H_2O) than that needed to open reabsorption atelectasis in anaesthesia (30 - 40 cm H_2O).[12,51-56] Recruitment maneuver studies can be divided into those using sustained or intermittent PEEP or PIP level increase, and those studies using special continuous PEEP settings or prone positioning. In all studies using single or repeated recruitment maneuvers that showed an increase in PaO_2 the effect was immediate but usually only short lasting.[57-68] Only one study on patients receiving mechanical ventilation for less than 72 hours showed a prolonged oxygen increase of 60% in 4 hours after a single

sustained inflation.[69]

In ARDS patients not responding to recruitment maneuvers, Grasso et al. found that a decreased chest wall compliance limited the transpulmonary gradient, and hence the driving force for recruitment,[57] a mechanism likely to explain the failure of recruitment maneuvers in non-responders in other studies.[61,70,71] Pelosi et al. observed that patients with primary ARDS were less responsive to a recruitment maneuver,[61] which is in concordance with the experimental studies by Kloot et al. mentioned before.[31] A special issue is ventilator disconnection or tracheal suctioning, which are common causes for desaturation. There are various studies that show that a closed suctioning system in combination with a recruitment maneuver consisting of sustained PIP elevation minimises de-recruitment, so here recruitment maneuvers seem to have a place.[72-74]

Several studies show that ventilation strategies using a continuous elevated PEEP level to increase alveolar recruitment lead to an increase in oxygenation, a finding that is compatible with the experimental studies.[62,66,68,75] This effect of PEEP increase on PaO_2 decreases at higher PEEP levels.[58,67,75,76] Increasing Vt by increasing PIP can also result in an increase in PaO_2.[66]

Notwithstanding the increases in oxygenation in the studies mentioned above, several large studies were unable to show any beneficial overall effect of recruitment maneuvers. Brower et al. studied 72 ARDS patients ventilated with Vt 6 ml/kg PEEP 13.8 cm H_2O (ALVEOLI trial) and showed that a recruitment maneuver using sustained PEEP resulted in a statistically significant but clinically minimal increase in oxygen saturation (1.7 ± 0.2% vs. 0.6 ± 0.3% in control patients with sham recruitment maneuver), but also a decrease in systolic blood pressure decrease.[76] The Canadian Open Lung Ventilation Study (pilot) using twice daily sustained PEEP increase to 35 cm H_2O was terminated after 28 patients were enrolled, as PaO_2 increased only 1.6 mm Hg, and four patients developed a pneumothorax, two severe hypotension and another four, ventilator dys-synchrony.[77] Furthermore, a randomised controlled trial in 549 ARDS patients comparing moderate (8.3 ± 3.2 cm H_2O) with higher PEEP levels (13.2 ± 3.5 cm H_2O) did not show a decrease in mortality or duration of ventilation.[78]

Prone positioning, reported for the first time in 1976 by Piehl et al.[79] has a special place in lung recruitment. Most studies found an improvement in oxygenation, although a substantial number of patients do not respond and the beneficial effect usually disappears after reversal to the supine position.[60,70,71,80-83,83-86] The increase in oxygenation in the prone position is explained by recruitment-related mechanisms such as improved ventilation through decreased alveolar compression by the heart and recruitment-independent mechanisms, e.g. a more homogeneous pleural pressure resulting in diminished intrapulmonary shunting mechanism.[71,84,87] The results of studies on FRC and end-expiratory lung volume (EELV) in prone position are

inconsistent [83,88] and, interestingly, chest compliance decreases in some studies.[71,83] As in other recruitment maneuvers, prone position might be less effective in late ARDS.[86] Although Stocker reported a lower mortality with prone positioning in the short term, a large randomised study including 304 patients by Gattinoni et al. did not show a statistically significant difference in mortality, although the power of this study might have been insufficient.[60,89] Serious complications have not been reported, apart from decubitus ulceration in less than 10%.[70]

Paediatric Anaesthesia and PICU (Table 3)

Although the lungs and chest in children are essentially different from those in adults (less alveoli, no alveolar interconnections, a maturing anti-inflammatory response to stress, increased chest wall compliance, relatively small role for gravitational forces), the general principles of recruitment and de-recruitment probably apply.[90] During anaesthesia a high FiO_2 also rapidly induces atelectasis in both assisted and spontaneous ventilation during sedation.[91,92] A single sustained inflation can reverse all atelectasis, the necessary PIP being between 25 and 30 cm H_2O,[93] which is slightly lower than the 40 cm H_2O reported in adults.[56,68,94,95] This may be explained by the increased chest compliance resulting in higher trans-pulmonary gradient for a given pressure. Contradictory results are reported as to whether PEEP without a recruitment maneuver can revert reabsorption atelectasis.[92,96] Although no paediatric studies exist on the preventive function of PEEP on atelectasis and shunting as it does in adults, a similar effect is likely.[55,56,94,95]

Studies on mechanical ventilation and recruitment maneuvers in the PICU are remarkably scarce, although most patients in the PICU require mechanical ventilation, and over 50% of them have PaO_2/FiO_2 ratios well below 200 mm Hg. No paediatric studies on optimal Vt, PEEP in relation to VILI and / or mortality have been published, but following the findings of the ARDS network trial, 6-8 ml/kg is generally adopted by most PICU as the optimal.[6] No paediatric studies exist on the long-term effects of recruitment maneuvers, although studies from the early 1990s show that clinically chosen PEEP is related to a pulmonary volume below FRC, and increasing PEEP levels until FRC is reached result in improved compliance, suggesting recruitment.[97,98] The short-term effects such as increase in oxygenation have been studied by several authors for prone position; the results being similar to those in the adult ICU: around 80% of ALI/ARDS patients respond to prone position with a significant increase in oxygenation, without reported negative effects on haemodynamics.[99-103] Numa et al. found that FRC was not affected by prone positioning, suggesting that pulmonary blood flow redistribution is more likely to explain the observed PaO_2 increase than alveolar recruitment.[101]

Table 2. *Anaesthesia and ICU studies on lung recruitment*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Cereda [73]	ALI n = 8	PEEP	Effect PEEP 5/10/15 on compliance	Vt 8.5	PEEP 15 stabilises compliance	
Ranieri [66]	ARDS n = 9	PEEP	Vt 10 vs Vt 5 ZEEP vs PEEP 10	PEEP 11 ± 1	Sat Vt10 > Sat Vt5 Sat PEEP > ZEEP	Cardiac index falls with PEEP in both Vt 5 and 10
Richard [75]	ARDS n = 15	PEEP	Vt 10/PEEP 11 vs Vt 6/PEEP 11 vs Vt 6/PEEP 15	PEEP 11 ("LIP")	Sat 96 Sat 95 Sat 96	
Dyhr [67]	Post cardiac surgery, n = 16	Sustained PIP	PIP 45 + PEEP 14 vs PIP 45 + ZEEP		Sat increases EELV increases	
Claxton [110]	Post cardiac surgery, n = 78	Combined PIP/ PEEP	A: ZEEP vs B: PEEP5 vs C: PIP 40/PEEP 5		A: sat = B: sat = C: Sat increase < 1 H	- - -
Brower [78]	ALI/ARDS n = 549	PEEP	PEEP 8.3 ± 3.2 vs 13.2 ± 3.5 cm H_2O			Mortality = ; MV duration =
Brower [76]	ALI/ARDS n = 96	Sustained PEEP	PEEP 35 for 30 s once daily in early ARDS	Vt 6, PEEP 13	Sat =	Systolic blood pressure decrease
Meade[77]	ALI ARDS n = 28	Sustained PEEP	PEEP 35-45 20-40 s 6x/3 days	Vt 6, PEEP >10	Sat =	4 pneumothorax 2 hypotension
Lapinsky [69]	ALI n = 14	Sustained PEEP	PEEP 30-45	PEEP 5-20	Sat increase 8% > 4 H	None
Richard [58]	ARDS n = 15	Sustained PEEP	A- Vt 10 vs Vt 6 B-Vt 6 + RM: sust PEEP 45	PEEP 11 ± 4 ("LIP") PEEP 15	A: Sat Vt 10 < Vt 6 B: Sat increase	
Povoa [64]	ARDS n = 8	Sustained PEEP	PEEP 25-45	Vt 6 PEEP 12 ± 3	Sat increase	None observed

Table 2. *(Continued)*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Grasso [57]	ARDS n = 22	Sustained PEEP	PEEP 40 for 40 s	Vt 6, PEEP	Sat increase only in early ARDS	
Villagra [59]	ARDS n = 17	Sustained PIP/PEEP	PIP 50, PEEP 30 for 2 min	PEEP 14 ± 1	Sat increase lasting < 15 min effect in early > late ARDS	Cardiac index =
Foti [62]	ARDS n = 15	Intermittent PEEP	PEEP 9 vs PEEP 16 vs PEEP 9 + RM: PEEP16	PEEP 13.3 ± 2.7 Vt 8	Sat PEEP16 > PEEP9 + RM > PEEP9	
Dyhr [68]	Cardiac surgery n = 30	Intermittent PIP	4 x PIP45 ZEEP vs 4 x PIP45 + PEEP12	Vt 5.7	Sat +, EELV+ < 5 min Sat +, EELV+ > 75 min	
Pelosi [61]	ARDS n = 10	sigh	2 hours baseline/1 hour intermittent PEEP 45/1 hour baseline	Vt PEEP 14 ± 2	Sat increase only during sigh	less effective in pulmonary ARDS
Patroniti [63]	ARDS n = 13	sigh	1 hour baseline/1 hour intermittent PEEP 35/1 hour baseline	PS 8-18 PEEP 11 ± 3	Sat increase only during RM	
Lim [65]	ARDS n = 20	sigh	Vt reduction to 0 PEEP increase to 30	Vt 8, PEEP 10	Sat and compliance increase	
Gattinoni [60]	ALI/ARDS n = 304	prone	Prone vs supine	PEEP 9 ± 3 Vt 10 ± 3	Sat increase	Mortality =
Stocker [89]	ARDS n = 25	prone	Prone positioning	not standardised		lower mortality
Blanch [82]	ARDS n = 23	prone	Rescue prone if PaO_2/FiO_2 < 200 mm Hg		PaO_2/FiO_2 increase Shunt decrease 66% responders	=

Table 2. *(Continued)*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Chatte [70]	ALI n = 32	prone	1 hour and 4 hours turning periods		Sat increase in PP 78% responders	desaturation in 6% decubitus ulceration
Douglas [80]	ARF n = 6	prone	Unchanged Vt, PEEP, FiO_2		PaO_2 increases with 70 mm Hg	
Pelosi [83]	ALI n = 16	prone	2 hours prone position		PaO_2 increase EELV =	Cch decreases
Pelosi [88]	ARDS n = 10	prone	Intermittent PEEP		Sat. incr, in PP > SP lasting > 1 hour	
Pappert [84]	ARDS n = 12	prone	2 hours prone		PaO_2 increase	In 33% PaO_2 decreases
Mure [81]	ALI n = 12	prone	A: supine B: prone	PEEP 7	A: Sat/FiO_2 1.06- B: Sat 1.50	
Lee [71]	ARDS n = 22	prone	12 hours prone, 2 hours supine	Vt 6-8 PEEP	PaO_2/FiO_2 increase Responders: 65%	Cch decreases large effect if large shunt
Guerin [85]	ALI/ARDS n = 12	prone	1 hour prone, 1 hour supine		PaO_2 increase	Cch decreases
Nakos [86]	Hydrostatic edema, n = 14 ARDS n = 20 Pulmonary fibrosis, n = 5	prone			Hydrostatic edema sat increase ARDS: 75% responders Pulmonary fibrosis minimal effect	Late ARDS: effect less than in early ARDS
Maggiore [72]	ALI n = 9	Sustained PEEP	PS 40 cm after suctioning		EELV loss less with PS-RM	
Dyhr [74]	ALI/ARDS n = 8	Sustained PIP	Sustained PIP 45 after suctioning		Sat decrease after suctioning, increase after RM	

Table 2. *(Continued)*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Neumann [95]	Anaesthetised n = 13	Sustained PIP	A: sust PIP 40 + ZEEP vs B: sust PIP 40 + PEEP 10.	FiO_2 1.0	A: atelectasis within 15 min atelectasis A > B PaO_2 A < B	
Rothen [56]	Healthy, Anaesthetised n = 12	Sustained PIP	sust PIP 40	FiO_2 0.4	PaO_2 increase atelectasis decrease	
Rothen [55]	Healthy, Anaesthetised n = 12	Sustained PIP			PaO_2 increased atelectasis diminished temporary VAQ diminished	
Rothen [53]	Healthy, Anaesthetised n = 12	-	A: FiO_2 0.3 vs B: FiO_2 1.0		PaO_2 A = B B: atelectasis increased B: VAQ-	
Rothen [54]	Healthy, Anaesthetised n = 12	Sustained PIP	Relation VAQ, atelectasis		Linear correlation shunt-atelectasis	
Tusman [94]	Healthy, Anaesthetised n = 30	Sustained PIP	A: ZEEP vs B: PEEP 5 vs C: Sust. PIP 40 + PEEP 5	FiO_2 0.4	A: atelectasis ++, PaO_2 18 B: atelectasis +, PaO_2 16 C: atelectasis -, PaO_2 24	
Tusman [111]	Healthy, Anaesthetised n = 12	Sustained PIP	Sust PIP in one-lung ventilation during surgery		PaO_2 increase +	

Vt: tidal volume in ml/kg; PS: pressure support in cm H_2O; Cch: chest compliance; see Table 1 for other abbreviations.

Table 3. *Paediatric studies on lung recruitment*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Curley [112]	ALI/ARDS n = 25 Age: 2 months to 17 years	prone	20 hours/day prone		PaO_2/FiO_2 increase	None
Kornecki [99]	ARF n = 10 Age: 8 weeks to 16 years	prone	12 hours prone vs. supine	Lung protective	PaO_2/OI increase Rrs/Crs =	None
Casado-Flores [100]	ARDS n = 23 Age: 0.5 months to 12.5 years	prone	Alternating per 8 hours		78% responders PaO_2/FiO_2 > 15%	Mortality 48% 80% in nonresp (n.s.)
Numa [101]	Obstr. lung dis n = 10 Restrict. lung dis n = 10 Control n = 10 Age: 3 to 7.5 years	prone	Prone vs. supine	 PEEP 2-6 PEEP 4 to 10 PEEP 2-4	 PaO_2+/FRC = PaO_2 =/FRC = PaO_2 =/FRC =	 Rrs + Rrs = Rrs =
Murdoch [102]	ARDS n = 7	prone	30 min prone/supine		PaO_2+/DO_2+	CO =/HR =
Sivan [97]	ARF n = 25 Age: 3 weeks to 10 years	PEEP	PEEP related to FRC		Clinically chosen PEEP < PEEP at FRC	

Table 3. *(Continued)*

Author	Study subjects	Recruitment Mode	Study design	Baseline ventilation	Outcome	Adverse effects
Sivan [98]	ARF n = 25 Age: 3 weeks to 10 years	PEEP	Crs related to FRC		Crs + at FRC > Crs below FRC	
Tusman [92]	Healthy infants n = 24 Age: 6 months to 6 years	PEEP	A: ZEEP vs B: PEEP 5 vs C: Sustained PIP 40 + PEEP 5		C: least Atelectasis	
Serafini [96]	Healthy infants n = 10 Age: 1-3 years	PEEP	Effect PEEP on CT image	FiO_2 0.4	ZEEP: < 5 min atelectasis PEEP: complete resolvement	
Marcus [91]	Healthy infants n = 20 Age: < 2 years	Sust PEEP	Sust PEEP 30 vs FiO_2 1.0		Rrs -, Compl + Rrs +, Compl -	
Sargent [93]	Healthy infants n = 32 Age: 12 to 62 months	PIP	PIP < 25 vs PIP > 30 vs SV with sedation		atelectasis PIP 25 > PIP 30 = SV	

FRC: functional residual capacity; OI: oxygenation index; DO_2: oxygen delivery; Rrs: resistance respiratory system; Crs: compliance respiratory system; CO: cardiac output; HR: heart rate; CT: computer tomography; n.s.: not significant; SV: spontaneous ventilation; see Table 1 for other abbreviations.

There are several studies that suggest that the infant lung might be more vulnerable to VILI than mature adult lung. Plotz et al. showed that only 2 hours of mechanical ventilation for cardiac catheterisation in otherwise healthy children led to an increase in pro-inflammatory cytokine response and decreased immunologic function of peripheral leucocytes.[104] In preterm infants the anti-inflammatory capacity is reduced, whereas the pro-inflammatory capacity is more matured, making the lungs more at risk for VILI.[105-107] Hence, studies performed in the adult ICU cannot automatically be extrapolated to the PICU population and, until PICU studies are available, results of ICU studies should be used with great care in the PICU.

Conclusion

As recruitment occurs during the entire ventilatory cycle, both PEEP and PIP elevations can result in increased oxygenation. However, an improvement in oxygenation is not necessarily related to a decrease in shear stress or VILI, and it is VILI that eventually determines outcome. Thus, increasing pressure levels to recruit pulmonary tissue should be guided by trials evaluating outcome. Evaluation of the literature shows that if recruitment maneuvers have an effect on oxygenation, this is usually short lasting and depends on the type and phase of underlying lung disease. Recruitment maneuvers are most effective in reabsorption atelectasis and de-recruitment due to MV disconnection or suctioning, and less effective in pneumonia and other conditions with decreased chest compliance like late ARDS. When sufficient PEEP is applied, as in most lung protective ventilation strategies, recruitment maneuvers are not effective. Prone position seems to be the safest and most efficient method for lung recruitment; however, no improvement on morbidity and mortality has been reported thus far. An effect of recruitment maneuvers on outcome remains unclear and they might be even harmful. Therefore the use of recruitment maneuvers should be patient-tailored and not routinely used until studies clarify the effect of recruitment maneuvers on oxygenation, VILI and morbidity / mortality. As paediatric diseases and physiology differ from adults, results from ICU studies need to be evaluated in the specific PICU setting.

Abbreviations

MV	mechanical ventilation
VILI	ventilator induced lung injury
LPVS	lung protective ventilation strategies
PICU	Pediatric Intensive Care Unit

PIP	peak inspiratory pressure
LIP	lower inflection point
PV-loop	pressure / volume-loop
FRC	functional resional capacity
EELV	end-expiratory lung volume
PEEP	positive end expiratory pressure
ICU	Intensive Care Unit
ALI	acute lung injury
ARDS	adult respiratory distress syndrome
PC	pressure control
PS	pressure support

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Chapter 6

Use of recruitment maneuvers during mechanical ventilation in Pediatric and Neonatal Intensive Care units in the Netherlands

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Introduction

Recruitment maneuvers (RM) during conventional mechanical ventilation (CMV) are increasingly advocated and seem to be widely employed, although beneficial effects of RM are unclear and the optimal strategy, indications, limitations, mode of evaluation and incidence of adverse effects are unknown.[1] These recruitment strategies vary from simple manual inflations to special mechanical ventilation (MV) settings. We investigated the use of RM during CMV in all Dutch Pediatric and Neonatal Intensive Care Units (PICUs / NICUs).

Material and methods

A written questionnaire concerning use, indications, monitoring, mode, applied pressures and adverse effects of RM was sent to all PICUs (n = 8) and NICUs (n = 10). RM were defined as any intervention intended to increase the number of alveoli participating in ventilation.

PICU size ranged from 6 to 27 beds with 285 - 1131 annual admissions; NICU size ranged from 12-28 beds with 233 - 539 admissions per year (*the Netherlands Perinatal Registry*). The answers were collected in September 2004.

Results

The response rate of this survey was 100%. Results of this survey are summarized in Table 1. RM are performed regularly in all PICUs and most NICUs, either manually using balloon (*PICU 100%, NICU 85%*), or mechanically by using the ventilator (*PICU 100%, NICU 57%*). These ventilator-assisted RM modes can be divided in isolated PIP elevation (8%), sustained PEEP elevation (25%) and combined elevation (58%). *Maximal applied pressures* appear substantially higher in PICUs than in NICUs: PEEP 28.3 ± 7.5 versus 9.2 ± 1.1 cm H_2O (p = 0.004), PIP 46.7 ± 12.1 versus 35.8 ± 4.9 cm H_2O (p = 0.02). One PICU had a formalized recruitment protocol, in the other units recruitment maneuvers appeared to be depending on the attending supervisor.

Reported *indications for RM* are poor oxygenation (*PICU 88%, NICU 85%*), atelectasis (*PICU 50%, NICU 43%*), high FiO_2 (PICU 25%, NICU 43%), and status after PEEP loss (tube disconnection, endotracheal suctioning) (*PICU 80%, NICU 46%*). Manual RM after PEEP loss is mostly done by nursing staff whereas ventilator-assisted recruitment maneuvers are exclusively performed by medical staff. *Efficacy of RM* is mostly evaluated by $TcSaO_2$ (*PICU 100%, NICU 100%*), less by PaO_2 (*PICU 25%, NICU 28%*), pressure-volume loop / minute ventilation measurements *(PICU 25%,*

Table 1.

Results of questionnaire *	**Overall (n = 18)**	**PICU (n = 8)**	**NICU (n = 10)**
Response	100%	100%	100%
Use of Recruitment Maneuver			
Never	17%	0%	13%
On indication	83%	100%	70%
Standard	0%	0%	0%
Indications			
Depending on underlying lung disease	0%	0%	0%
Depending on clinical symptoms	100%	100%	100%
Poor saturation	87%	87%	86%
High FiO_2	33%	25%	43%
Atelectasis	47%	50%	43%
After PEEP loss (endo-tracheal suctioning / Ventilator disconnection)	60%	75%	43%
Mode of Recruitment Maneuver			
Manual RM	93%	100%	85%
with pressure measurement	43%	13%	83%
without press. measurement	57%	88%	17%
RM with ventilator	80%	100%	57%
Protocol	7%	13%	0%
Sustained PEEP	8%	0%	25%
Sustained PIP	25%	25%	25%
Intermittent PEEP	8%	0%	25%
Intermittent PIP	0%	0%	0%
Combination	58%	75%	25%
Prone position	87%	88%	85%
Mode of evaluation			
Transcutaneous Saturation	100%	100%	100%
Blood gas analysis	27%	25%	29%
Compliance / pressure volume loops / lung volume measurements	27%	25%	29%
Chest X-ray	40%	13%	71%
Adverse effects			
Increase of VILI	0%	0%	0%
Pneumothorax / pneumatocèle / emphysema	0%	0%	0%
Significant hemodynamic changes	40%	50%	29%
Oxygenation decrease	20%	18%	22%

* expressed as percentage of centers on which question is applicable

NICU 28%), chest X-rays (*PICU 25%, NICU 71%*). *Adverse effects* reported are blood pressure decrease and oxygen desaturation (*PICU 50%, NICU 28%*); no gross barotrauma (pneumothorax, pulmonary emphysema) has been reported.

Discussion

These data show that in the Netherlands RM are used in all PICUs and most NICUs. However the obtained results also show a large diversity, corresponding with numerous publications on RM with different strategies and inconsistent results, which we reviewed extensively.[1]

A combined RM mode with both PIP and PEEP elevation, as used by most centers, is theoretically the most effective mode as recruitment and derecruitment are continuous processes throughout the ventilatory cycle, during which PIP recruits alveoli and PEEP prevents alveolar re-collapse.[2] An isolated PIP increase - e.g. manual RM - has the risk of alveolar overdistention and increased shear stress forces in not stabilized alveoli, leading to possibly lung injury. Sustained elevation of PEEP level seems less injurious and increases pulmonary aeration temporary in experimental studies.[3] Only after MV disconnection or endotracheal suctioning, temporary PIP increase is rational as this rapidly recruits collapsed alveoli [4] and repeated derecruitments appear to be harmful.[5] Prone positioning, as widely used by 87% centers, appears to increase oxygenation in many studies by decreasing pulmonary compression by the heart and improving ventilation-perfusion match.[6] However, as in adults, the effect usually disappears after repositioning.[7,8] The only large study evaluating prone position in adults (n = 304) showed a non-significant difference of 4% on mortality, but this study was probably underpowered.[9]

Several studies indicate that in late phase of respiratory failure RM rarely improve oxygenation.[1,10] Interestingly, phase of disease was not reported to influence RM indications in any of these centers, nor was the cause of underlying disease, although one centered mentioned obstructive lung disease as contra-indication. Only ventilatory parameters ($TcSaO_2$, FiO_2) were indicated.

The maximal reported RM pressures - in PICUs significantly higher than in NICUs - are similar to those applied in clinical studies (Figure 1).[1] Recruitment pressures needed to open alveoli depend on lung condition, being in the diseased lung substantially higher (up to 45 cm H_2O in ARDS) [11] than in healthy lungs, in which 25 - 30 cm H_2O - and in low birth weight infants probably less - is sufficient.[12,13] Interestingly, use of pressure manometers on balloons as safety device varied substantially (*PICU 12.5%, NICU 90%*). The parameters commonly used to evaluate the effect of RM, $TcSaO_2$ and PaO_2, reflect well the degree of intrapulmonary shunting

caused by non-ventilated alveoli; minute-ventilation and chest X-ray in CMV are less suitable. Absolute pulmonary volume measurements may become more important in future studies on lung recruitment, but at present it is still technically difficult. For pressure / volume (PV) loop analysis there is a limited role in establishing optimal recruitment, as it is greatly influenced by external factors like chest wall compliance and abdominal pressure, and lung history.[14-16]

The encountered adverse effects on hemodynamics are consistent with those reported in the literature.[17;18] However, despite the absence of reported adverse effects on lung injury, and possible advantages of RM, a critical review of the literature regarding both short term effects - oxygenation, hemodynamics - and long term parameters - morbidity and lung injury - does not support the routine use of RM.[1] In a large clinical RM study (n = 72) a temporary clinically irrelevant increase in $TcSaO_2$ (1.7%) was found.[19] The Canadian Open Lung Ventilation pilot study, using thrice daily sustained PEEP elevations to 35 cm H_2O was aborted as there was minimal improvement of oxygenation and serious adverse effects occurred.[20]

Conclusion

Although the use of RM during conventional MV is apparently widespread and no large adverse effects are encountered bedside, in our opinion RM should still be considered carefully regarding the lack of evidence.

Abbreviations

RM	recruitment maneuvers
CMV	conventional mechanical ventilation
MV	mechanical ventilation
PICU / NICU	Pediatric Intensive Care Unit / Neonatal Intensive Care Unit
PIP	peak inspiratory pressure
PEEP	positive end expiratory pressure
$TcSaO_2$	trans cutaneous oxygen saturation
ARDS	adult respiratory distress syndrome
PV-loop	presuure volume loop

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Chapter 7

A single Recruitment Maneuver in ventilated children translocates cytokines into the circulation

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Abstract

Introduction: Recruitment maneuvers (RM) are frequently advocated to prevent pulmonary collapse during low tidal volume ventilation and to improve oxygenation. However, recent experimental studies also suggest various adverse effects of RM such as translocation of inflammatory mediators from the alveolar space into the circulation. To determine whether a single RM in ventilated children affects pulmonary and systemic cytokine levels we performed a prospective intervention study .

Methods: Cardio-respiratory stable patients (0.5 - 45 months, n = 7) requiring mechanical ventilation for Acute Lung Injury (PaO_2 / FiO_2 < 300 mm Hg) in an Academic Paediatric Intensive Care Unit were subjected to a recruitment maneuver consisting of combined increase in PIP (maximally 45 cm H_2O) and PEEP (maximally 30 cm H_2O) while ventilating with a tidal volume of 6 ml/kg. Before and after RM cardio-respiratory parameters and ventilator settings were recorded and blood gas analysis performed. In broncho-alveolar lavage fluid and plasma TNF-α, IL-1β, IL-6, IL-8 and IL-10 concentrations were determined before, and 15, 60 and 360 min after the RM. Data were expressed percentage increase of baseline values.

Results: 15 minutes after the RM, an increase was observed in plasma TNF-α (400 ± 390%, $p = 0.04$), IL-6 (120 ± 35%, $p = 0.08$) and IL-1β (520 ± 535%, $p = 0.04$), with subsequent decrease to baseline levels at T = 60 minutes, hence indicative of tranlocation. The RM did not change the plasma levels of the anti-inflammatory IL-10 (105 ± 12%, $p = 0.5$). Apart from a non-significant increase of IL-8 after 360 minutes (415 ± 590%, $p = 0.1$), broncho-alveolar cytokine levels were not influenced by the RM. No increase in oxygenation, or improvement of lung kinetics was observed.

Conclusions: A single recruitment maneuver translocates pro-inflammatory mediators from the alveolar space into the systemic circulation in ventilated critically ill children.

Introduction

Mechanical ventilation with low tidal volumes (Vt) reduces patient mortality in acute respiratory failure.[1,2] However, small tidal volumes (< 6 ml/kg) can result in loss of aerated lung tissue and oxygen desaturation. To augment the number of aerated alveoli, various lung recruitment maneuvers (RM) have been developed and are commonly used.[3] Although several experimental studies show a positive effect of RM on oxygenation, clinical studies show controversial results.[4] In patients ventilated with sufficient PEEP and low Vt, RM appear to improve oxygenation in only a minority of patients which generally lasts only for a short period of time.[4-6] Recently use of RM has been discouraged in ARDS by Girard et al.[7]

Both animal and clinical studies have shown that mechanical ventilation with high airway pressures is associated with elevation of cytokine levels and lung injury. Increase of ventilation pressures can augment the levels of inflammatory markers within 1 hour in adult patients with Acute Lung Injury (ALI).[8,9] Moreover, translocation of inflammatory mediators and bacteria from the alveolar space into the systemic circulation after a high pressure recruitment maneuver, has been demonstrated in several experimental models, and these elevations are associated with lung injury and multi-organ failure.[10-12] These results illustrate the possible deleterious effects of RM.

Recruitment studies in healthy children are rare and non-existent in critically ill children.[4] Although the general principles of recruitment and derecruitment in the adult patient probably also apply to small children, lungs and chest wall are essentially different, e.g. decreased number of alveoli, increased chest wall compliance and a relatively small role for gravitational forces.[4,13] Also, the maturing inflammatory response to stress is essentially different from adults.[14,15] Hence, observations in adult ICU patients cannot directly be extrapolated to critically ill children.

In the current study we determined the effect of a single, RM in children on the pulmonary and systemic inflammatory response.

Material and methods

Both the hospital- and the national ethical committee approved the study. Written informed consent was obtained from both parents before inclusion.

Study population

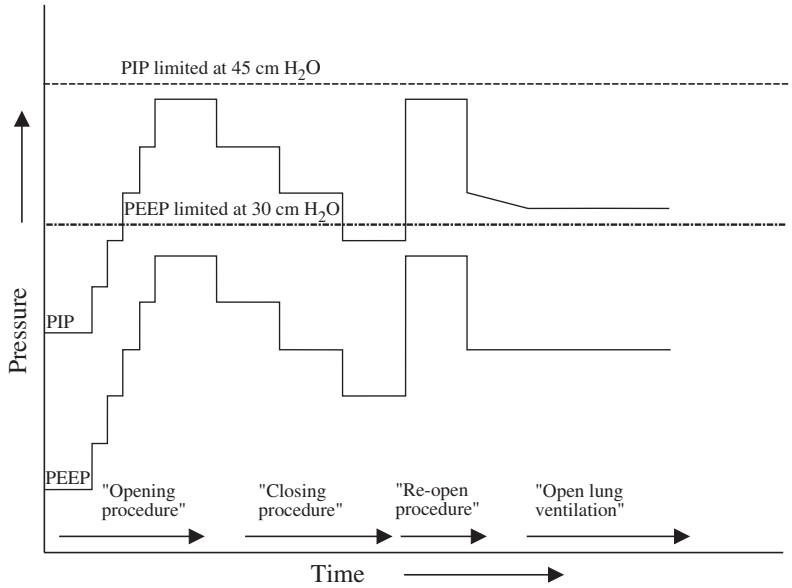
Inclusion criteria for this study were Acute Lung Injury (ALI) as defined by the American European Consensus Conference Definition: PaO_2/FiO_2 < 300 mm Hg (40kPa), bilateral pulmonary infiltrates on chest X-ray consistent with edema without clinical signs of cardiac failure.[16] As the inflammatory response matures with gestational age (GA) only patients with a GA over 37 weeks were included.[14] Patients were included within 72 hours after the diagnosis of ALI.[5,17] Exclusion criteria were risk factors for barotrauma (baseline peak inspiratory pressure (PIP) > 35cm H_2O, pneumothorax, pneumatocele, bronchopleural fistula, recent pulmonary surgery), risk factors for hemodynamic compromise (Fontan circulation, intracardiac shunt, severe hemodynamic instability defined as the need for norepinephrine > 0.2 μg/kg/min despite adequate fluid therapy) and medication interfering with the study design (corticosteroids or other anti-inflammatory drugs).

RM procedure

Prior to a RM, patients were ventilated with a Siemens Servo 300® ventilator in supine position with adequate sedation. All patients were clinically euvolemic. We used a modified RM according to Hickling and Villagra[17,18] (Figure 1). Ventilation mode was switched to Pressure Control (PC) 15 cm H_2O above PEEP and FiO_2 adjusted to obtain a transcutaneous oxygen saturation ($TcSaO_2$) of 90%, corresponding with maximal PaO_2 change on the oxygen-dissociation curve. PEEP and PIP were then increased with 2 cm H_2O every 5 - 10 seconds until $TcSaO_2$ reached ≥ 98%, indicating maximal recruitment ("opening pressure"). The maximal PEEP and PIP level allowed was 30 and 45 cm H_2O, respectively. Subsequently, PEEP and PEEP were lowered with 2 cm H_2O per 5 - 10 seconds until $TcSaO_2$ started to decrease, suggesting lung derecruitment ("closing pressure"). To re-open the lung PEEP and PIP were again increased to the previously determined opening pressure for 30 seconds after which PEEP and PIP were lowered and maintained at the level obtained one step above "closing pressure". Subsequently, PIP was adjusted to obtain a Vt 6 ml/kg.

At T = 0 (before RM), and T = 15, T = 60 and T = 360 minutes after RM we recorded arterial blood pressure, heart rate and if applicable central venous pressure together with PIP, PEEP, Mean Airway Pressure, Vt, respiratory rate and FiO_2.

Figure 1.



Recruitment Maneuvre: "Opening procedure " PIP at 15 cm H_2O PEEP, increase 2 cm/5 seconds till $TcSaO_2 \geq 98\%$ or PIP ≥ 45 / PEEP ≥ 30 cm H_2O; "Closing procedure": PIP / PEEP reduction 2 cm/15 seconds till $TcSaO_2 << 90\%$; "Re-opening procedure": 30 seconds PIP/PEEP reset at level reached during "opening procedure". "Open lung ventilation": PEEP at lowest level with $TcSaO_2 > 90\%$, PIP reduced till Vt = 6 ml/kg.

Inflammatory parameters

Samples were obtained from an arterial canula at T = 0, 15, 60, 360 min for blood gas analysis and measurements of inflammatory parameters. Tracheo-bronchial aspiration was performed at T = 0, 15, 60, 360 min as described previously.[9,19] In summary, a 6 - 8 F suction catheter (Vygon, France) was advanced through the endotracheal tube until the catheter reached the wedge position. Subsequently, 1 ml NaCl 0.9% was instilled and immediately aspirated.

Laboratory analysis

Plasma and tracheo-bronchial lavage fluids were processed by immediate centrifugation at 500 G at 4° C for 15 minutes and then stored at -80° C prior to analysis. Concentrations of TNF-α, IL-1β, IL-6, IL-8 and IL-10 were determined using simultaneous Luminex assay.[20] Detection limits were for TNF-α 150 pg/ml, for IL-1β 20 pg/ml, for IL-6 5 pg/ml, for IL-8 20 pg/ml and for IL-10 5 pg/ml.

Statistical analysis

The primary end point was the effect of a RM on the concentration of circulating cytokines. We planned to include 10 patients. Since this pilot study investigates the potential deleterious effects of RM, an interim analysis was planned after inclusion of 5 children. To correct for inter-individual variance, cytokine concentrations were expressed as a ratio against their respective baseline values at T = 0 min. (e.g. ratio at T = 15 min: ConcentrationT = 15 / ConcentrationT = 0) Concentrations of BAL cytokines were not corrected for aspirated volume or an inert marker, consistent with advise from European Respiratory Society.[21]

Statistical analysis was performed using one sided paired Student t-test, as the objective was to demonstrate whether plasma levels increased as the result of translocation and we had no interest in formally demonstrating the opposite alternative. All data are expressed as mean ± SEM unless specified otherwise. Graphs are composed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA.

Results

After the inclusion of 5 patients, an interim analysis was performed, during which two additional patients were included. When the cytokine data became available, the study was terminated. Median age was 6 [range: 0.5 - 45] months, admission diagnosis was meningococcal sepsis (n = 2) , RSV bronchiolitis (n = 2), Influenza A pneumonia (n = 1), respiratory insufficiency with hypovolemic shock and viral sepsis (n = 1), and pneumonia (n = 1).

Mean PIM and PRISM scores were -2.9 ± 1.7 and 13.0 ± 8.4, respectively. Admission PaO_2/FiO_2 ratio was 25.9 ± 10.9 kPa, indicating significant pulmonary dysfunction. Median duration of ventilatory support after ALI was 6 [3 - 28] days.

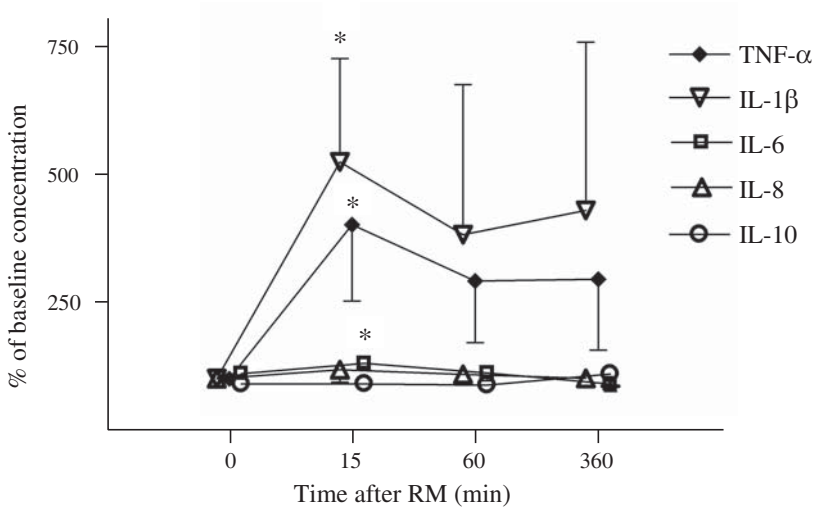
Effects of RM on systemic inflammatory parameters

Cytokine plasma levels at T = 0 min exhibited a large inter-individual variance: TNF-α 67 ± 58 pg/ml, IL-1β 19.6 ± 10.5 pg/ml, IL-6 78.1 ± 37.5 pg/ml, IL-8 58.6 ± 29.5 pg/ml, IL-10 30.7 ± 29.6 pg/ml. A single RM induced a 400 ± 390 % increase in TNF-α ($p = 0.04$), 120 ± 40% increase in IL-6 ($p = 0.08$) and 520 ± 530% elevation in Il-1β ($p = 0.04$) at T = 15 min, followed by a subsequent decrease to baseline values (Figure 2).

Effects of RM on pulmonary inflammatory parameters

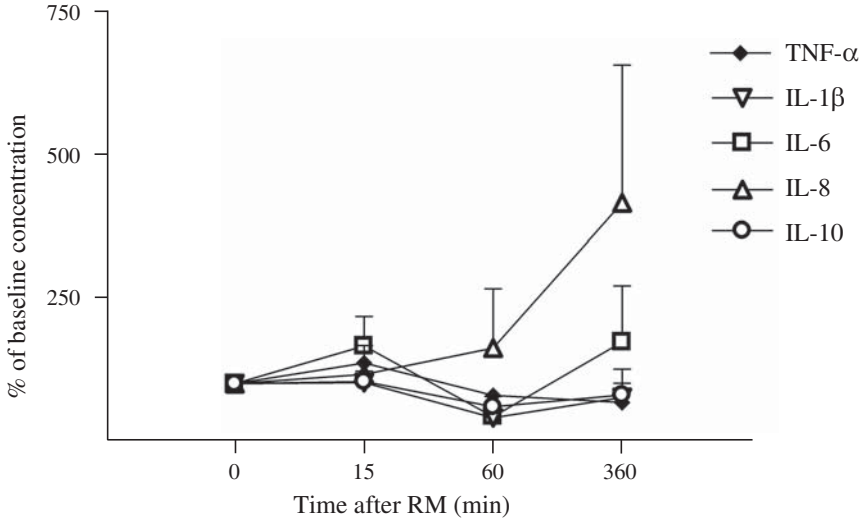
Tracheo-bronchial Cytokine levels at T = 0 min exhibited a large inter-individual

Figure 2.



Change in plasma cytokine levels, as ratio of initial concentration after a single recruitment maneuver (RM) (T = 0). Data expressed as mean ± SE. After RM a temporary increase is observed in plasma TNF-α (400 ± 390%, $p = 0.04$), IL-6 (120 ± 35%, $p = 0.08$) and IL-1β (520 ± 535%, $p = 0.04$).

Figure 3.



Change in Broncho-Alveolar Lavage fluid, as ratio of initial concentration after a single recruitment maneuver (RM) (T = 0). Data expressed as mean ± SE. After RM a non-significant change in IL-8 at T = 360 is observed ($p = 0.1$)

variance: TNF-α 28 ± 64 pg/ml, IL-1β 408 ± 393 pg/ml, Il-6 663 ± 674 pg/ml, IL-8 38150 ± 45757 pg/ml, IL-10 106 ± 145 pg/ml. A single RM did not result in significant changes in BAL concentrations of the measured cytokines. However, there was a trend of increasing IL-8 levels at T = 360 min (p = 0.1), (Figure 3).

Effect of RM on $TcSaO_2$ and hemodynamics

Complete recruitment, with oxygen saturation ≥ 98% was achieved in 1/7 patients, incomplete recruitment ($TcSaO_2$ 92 - 97%) was observed in 1/7 patients. No increase in oxygenation was observed in the remaining 5 patients. Mean PIP during RM was 39.7 ± 4.3 cm H_2O, mean PEEP during RM 25.2 ± 5.3 cm H_2O, mean maximal $TcSaO_2$ was 93.3 ± 3.0%, mean minimal $TcSaO_2$ 84 ± 4.1%.

Hemodynamic deterioration during RM occurred in 2/7 patients. One patient needed an i.v. fluid bolus. All cardio-respiratory parameters normalized over time.

Ventilatory support and blood gas analysis.

RM did not significantly change PaO_2 (from 11.9 ± 2.6 to12.4 ± 2.4 kPa), $PaCO_2$ (from 5.0 ± 0.8 to 4.7 ± 0.9 kPa) or pH (from 7.39 ± 0.06 to 7.41 ± 0.1) (all $p > 0.1$). Also, no significant changes before and after RM in PaO_2/FiO_2 ratio (from 25.9 ± 10.9 to 23.2 ± 8.7 kPa), Oxygenation Index (4.6 ± 2.5 to 4.6 ± 2.9), PIP (from 21.9 ± 3.9 to 20.1 ± 3.6 cm H_2O) or PEEP pressure (from 6.3 ± 2.0 to 6.1 ± 2.1 cm H_2O) were observed (all $p > 0.2$).

Discussion

Our study shows that a relatively mild and widely used method to recruit alveoli 3 results in a transient increase in circulating cytokines in ventilated critically ill children. The rapid and transient increase indicates that increased up-regulation of cytokine production is not the primary responsible mechanism, since this would take far longer than the 15 minutes to reach the observed peak levels. From these data we conclude that the RM increased circulating cytokines by translocation of pulmonary cytokines into the systemic circulation.

We deliberately selected a recruitment procedure with a small pressure gradient between PIP and PEEP to examine whether or not this safe RM resulted in cytokine translocation.[4,22-25] A national survey in all Dutch pediatric and neonatal ICUs by us showed that this RM mode is the most frequently used, emphasizing the clinical relevance of our study.[3]

Our observation that inflammatory mediators from the alveolar space may enter the systemic circulation after a single RM likely represents clinical importance, even the

short duration and magnitude. TNF-α appears to be involved in the pathogenesis of both Ventilator Induced Lung Injury (VILI) and Multi Organ Dysfunction syndrome and prolonged elevation of systemic cytokines is associated with increased morbidity. This indicates a relation between mechanical ventilation and multi organ dysfunction syndrome, as suggested by several authors.[11,26-29] Thus the observed translocation of TNF-α, IL-1β and IL-6 from the alveolar space into the systemic circulation may contribute to the development of multi organ dysfunction syndrome[30-32] when RM are routinely and frequently applied. This is consistent with *in-vivo* observations which show that mechanical ventilation increases alveolar-capillary permeability [33] and that superimposed RM can translocate cytokines and bacteria [11,29] into the systemic circulation.

The balance between the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8 and the anti-inflammatory cytokines such as IL-10 appears to be essential for directing the immune response.[34] In our study the pro-inflammatory cytokines were translocated by the RM, whereas no effect on the anti-inflammatory cytokine IL-10 was observed.

The increased IL-8 levels in BAL fluid at T = 360 min after the RM, though not statistically significant, possibly indicates increased alveolar inflammatory activity. IL-8 is known to be involved in the pathogenesis of injurious mechanical ventilation[34-38] and a strong chemo-attractant for polymorphonuclear neutrophilic granulocytes, the cells that appear to be a pre-requisite for lung injury.[39,40]. The increase in pulmonary IL-8 in the absence of a systemic increase suggests alveolar up-regulation rather than translocation or decompartmentalization into the alveolar space. It is unlikely that tracheo-bronchial aspiration with a small volume of 1 ml NaCl 0.9% evokes the observed changes.

Our observations are comparable with results obtained in animal studies using higher inspiratory pressure.[10] In contrast, a recent clinical study in adults with atelectasis predominantly admitted for cerebral pathology, a RM with a pressure of 40 cm H_2O for 7 seconds was not found to induce translocation of cytokines.[41] The differences with our study may be explained by better maintained alveolar-capillary integrity in that population, and by the fact that their RM mode lasted substantially shorter than the one we used. We propose that the magnitude of cytokine translocation probably depends on both the applied transpulmonary pressure and the degree of alveolar-capillary permeability. To our knowledge, our data represent the first study concerning the effects of RM in ventilated children and suggests that potentially harmful effects of a RM may be more pronounced in this group of patients compared to adults.

The number of included patients is relatively small, however the interim analysis after inclusion of 5 patients showed significant cytokine elevation, which was even more pronounced when 2 patients included during this interim analysis were added. As

determined beforehand, the study was terminated when these results became available. The relatively limited number of patients and varying underlying diseases and pulmonary dysfunction possibly accounts for the observed differences in response to RM. Nevertheless, we did not find different patterns in the release of cytokines between the patients with a pulmonary infection compared to those with a non-pulmonary cause of ALI (data not shown).

The observation that our RM increased $TcSaO_2$ in only in a minority of patients is consistent with the adult literature although more favourable data also exist.[4,42] In adult ARDS, use of RM has been discouraged recently.[7] No data on RM in paediatric ICU patients are available, and despite that the effect of RM is debatable[4] they are still considered safe.[43] Airway pressures may have been too low to obtain a significant improvement in oxygenation. As discussed above, we were not aiming to demonstrate cytokine release by more vigorous RM methods and well-considered selected a widely used and relatively mild RM method. In our view, it is likely that higher pressures may increase cytokine translocation even further.

Conclusion

Despite possible short-term beneficial effects on oxygenation, RM in ventilated small children result in an increase in circulating cytokines and may contribute to ventilator associated lung injury and multi organ dysfunction syndrome by translocating inflammatory mediators from the alveolar space into the systemic circulation. Randomized controlled trials are needed before recruitment procedures are generally accepted in clinical care.

Abbreviations

Vt	tidal volumes
RM	recruitment maneuvres
ALI	acute lung injury
GA	gestational age
PIP	peak inspiratory pressure
PC	Pressure Control
$TcSaO_2$	transcutaneous oxygen saturation
Bal	Broncho-alveolar lavage
VILI	Ventilator Induced Lung Injury

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Chapter 8

The oxygenation ratio during mechanical ventilation in children: *the role of tidal volume and PEEP*

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Abstract

Objective: To analyze the role of tidal volume (Vt) and PEEP on the Oxygenation Ratio (OR: PaO_2 / FiO_2) during mechanical ventilation (MV) in children with a normal pulmonary gas exchange on admission.

Methods: Retrospective cohort study of children with an admission OR > 300 mm Hg and duration of MV > 48 hours (n = 96). We analyzed Vt, FiO_2, PaO_2 and PEEP and calculated Vt (ml/kg) and PaO_2 / FiO_2 based on measured Vt and weight. Patients were divided in group I: Vt < 9 ml/kg (n = 24), II: Vt 9 - 12 ml/kg (n = 58), and III: Vt ≥ 12 ml/kg (n = 14).

Results: Baseline characteristics and OR were comparable. 41% of patients developed OR < 300mm Hg. The proportion of patients developing an OR < 300 mm Hg was lowest in group I and highest in group III and differences became more pronounced with longer MV duration: 56%, 58%, 89% on day 5; 29%, 65%, 100% on day 7 (p = 0.05); 0%, 40%, 100% on day 10 (p = 0.03). In patients maintaining a OR > 300 mm Hg during 10 days of mechanical ventilation Vt was 9.3 ± 1.0 ml/kg versus 12.7 ± 4.8 ml/kg in patients developing a OR < 300 mm Hg (p = 0.05). MV duration was longer in children developing OR < 300 mm Hg (p < 0.01). PEEP levels were not significantly different between groups.

Conclusion: In ventilated children, tidal volumes > 9 ml/kg were associated with increased development of an OR < 300mm Hg and longer duration of MV.

Introduction

Mechanical ventilation (MV) may be an important factor contributing to lung injury and mortality in adult patients admitted to the Intensive Care Unit (ICU).[1,2] High tidal volumes (Vt), high inspiratory pressures and low Positive End Expiratory Pressure (PEEP) levels are thought to be the main determinants contributing to *ventilator associated lung injury* (VALI) through a process of hyperinflation and shear stress induced inflammation, surfactant dysfunction and reduced lung fluid clearance (3-8). Reducing Vt to 6 ml/kg decreases VALI and improves survival in ARDS patients compared to a Vt of 12 ml/kg.[1,9] Also, in adult patients without lung injury on ICU admission, development of acute lung injury (ALI) appears to be associated with the use of Vt > 6 ml/kg.[2]

As there are major differences between children and adults lungs, results obtained from ventilated adults cannot be extrapolated directly to the critically ill child. Lung anatomy changes with aging: total number of alveoli still increases until the 3rd year of life, whereas inter-alveolar (Kohn's) pores, which facilitate collateral ventilation and may reduce alveolar overinflation, develop only after the age of 4 years.[10,11] Compliance in the infant is much higher, predominantly due to high compliance of the thoracic cage, making the infant lung more vulnerable to pressure increase.[12] Also, functional residual capacity is lower in children, and a lower lungweight / bodyweight ratio implies that in children a given Vt results in larger Vt/gram lungtissue than in adults.[13] Furthermore, the inflammatory response and development of innate immunity, involved in the pathogenesis of VALI, develops during fetal and neonatal life, with the anti-inflammatory response maturating slower, resulting in a predominantly pro-inflammatory balance during stress.[14,15]

In children, the optimal Vt resulting in adequate ventilation and minimizing VALI is unknown, as no randomized controlled trials exist in this population. Therefore, we analyzed applied Vt and PEEP in children admitted with a normal oxygenation ratio (OR: PaO_2 / FiO_2) of > 300 mm Hg, and related these factors to the subsequent development of OR. The results of this retrospective cohort study suggest that the use of higher tidal volumes contributes to the development of a oxygenation ratio < 300 mm Hg.

Methods

We performed a retrospective observational cohort study of all children admitted between January 1 2003 and December 31 2004 (n = 1091) to our pediatric intensive care unit (PICU) in St Radboud Nijmegen University Centre. Following Dutch law, approval by an Ethics Committee was exempt for this retrospective observational study. Demographic data, admission diagnosis, PaO_2, $PaCO_2$, pH, MV duration FiO_2, Vt, PEEP, Peak Inspiratory Pressure (PIP), and Mean Airway Pressure on day 1, 5, 7 and 10 were retrieved from our Pediatric Index of Mortality (PIM) and Pediatric Risk of Mortality (PRISM) score databases and the patient's medical charts.[16,17] From these data OR and Vt (ml/kg) body weight were calculated.

Patients were included if their OR was > 300 mm Hg (40 kPa) on admission (independent of PEEP and FiO_2), and MV duration lasted for ≥ 48 hours (n = 104). Out of these 104 children, 8 were excluded as data were either incomplete (e.g. only capillary or venous blood gas, (n = 3), irretrievable (n = 1), or obviously incorrect entered in the database (n = 4). Between day 5 and day 10 nine more children were excluded, 7 of them because only venous blood gas analysis was available due to removal of the intra-arterial catheter and 2 due to incorrect data entry in the database. Patients were also excluded when they developed a complication directly affecting OR other than VALI, i.e. ventilator associated pneumonia (VAP),lung embolism, cardiac failure, transfusion related acute lung injury (TRALI). Patient data were excluded from analysis from day of complication diagnosis onwards. VAP diagnosis was adapted from CDC criteria.[18]

According to the European Consensus Conference Definition for acute lung injury in adults, an OR < 300 mm Hg was chosen to define significant gas exchange dysfunction consistent with VALI.[19]

We checked for patients with clinical, echo-cardiographic or measured signs of increased left atrial pressure and embolism, however they did not occur in this data set. The two other criteria of the adult ALI definition (left atrial pressure ≥ 18 cm H_2O and new pulmonary infiltrates on chest X-ray) are not validated in children and thus we describe the observed decrease in OR as VALI rather than ALI.

We restricted the analysis period to 10 days, since only 9 children were still ventilated from day 11 onwards. Extubation criteria were left to the attending physician, but generally included circulatory stability and adequate neurological function in addition to normalizing OR.

Prior to analysis and based on results of previous studies in adults,[1,2] patients were divided in three groups: Group I Vt < 9 ml/kg, Group II Vt 9 - 12 ml/kg and Group III Vt ≥ 12 ml/kg. We analyzed the effects of admission Vt and average Vt on the fraction of patients developing OR < 300 mm Hg in both ventilated children as well as in the whole cohort including extubated patients. We only analyzed initial (and not averaged) PEEP levels as PEEP levels during later stages of mechanical ventilation were likely adjusted to the changing OR.

In a second analysis, we divided the total group of patients in two subgroups: no development of VALI with OR remaining > 300mm Hg and patients developing an OR < 300mm Hg. In these two groups, Vt and PEEP was compared.

The actual delivered Vt (measured by ventilator reading, Servo 300®, Siemens, Munchen, Germany) was used for the calculations and not the prescribed Vt, as we wanted to use data as they were applied in clinical practice. No correction was made for compressible volume. We included children of different ethnic origin and with syndromal anomalies, therefore no correction was made for ideal body weight. No children with morbid obesity were encountered in this cohort.

Statistical analysis

Demographic data are given as mean ± SD or as median [range], depending on their distribution. A Chi-square test was used to analyze categorical data, or Fisher Exact test when appropriate.

For continuous data of OR groups, two-sided Student's t-test or Wilcoxon rank test was performed depending on their distribution; for analysis of 3 Vt groups ANOVA or Kruskall-Wallis was used depending on their distribution (SPSS 13.0, SPSS Inc. Chicago, IL, USA).

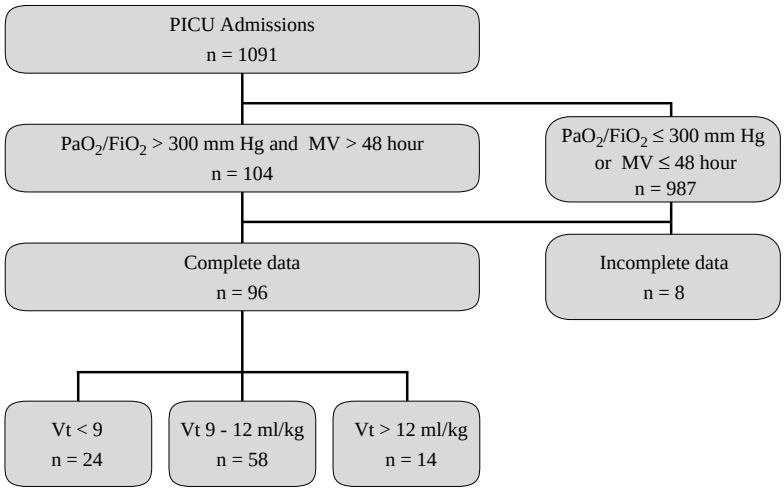
Results

Patient characteristics

Figure 1 illustrates the inclusion and exclusion of patients, and subsequent division in Vt groups. The included children (52 male, 44 female) had a median [range] age of 2.1 [0.01-16.1] years. Demographic data, admission diagnoses and disease severity scores, blood product transfusion data, blood gas analysis, and ventilator settings are shown in Table 1 and 2.

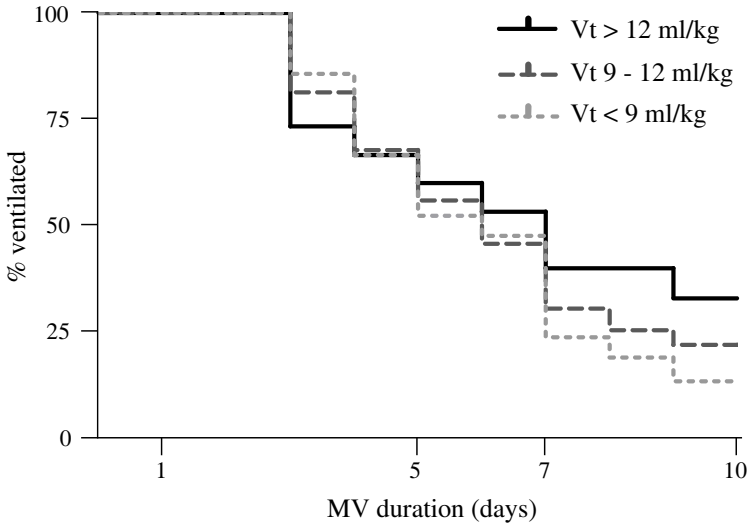
VAP was diagnosed in 7 patients on day 5 increasing to 18 patients on day 10; patients with VAP were distributed equally among groups I, II and III (p = 0,47) and groups with OR < and > 300 mm Hg (p = 0,19).

Figure 1.



Study Flow Diagram.

Figure 2.



Percentage of included patients being ventilated without VAP during observation period.

The proportion of group I, II and III being ventilated without VAP during the observation period is illustrated in Figure 2.

Vt on admission (10.2 ± 2.2 ml/kg) and average Vt during mechanical ventilation were comparable (10.1 ± 2.5 ml/kg). Included patients had a mean OR of 432 ± 138 mm Hg on admission. PEEP level on admission was 4.9 ± 2.5 cm H_2O.

Table 1. *Admission characteristics of group I, II, III*

		Group I Vt < 9 ml/kg	**Group II Vt 9 - 12 ml/kg**	**Group III Vt > 12 ml/kg**	***p*-value**
Sex M/F	%	54 / 46	55 / 45	50 / 50	0.89
Age (years)	Median [range]	8.3 [0.1-15.6]	1.8 [0.1-16.1]	0.6 [0.1-11.4]	< 0.01
PRISM score	Mean ± SD	18 ± 8.2	20 ± 6.2	16 ± 5.7	0.65
PIM score	Mean ± SD	2.5 ± 2.7	2.4 ± 1.2	2.1 ± 2.2	0.43
Admission diagnosis					
Respiratory tract disease	%	4	10	7	0.64
Sepsis	%	21	21	29	0.80
Post-surgical	%	50	44	37,5	0.75
Neurologic dysfunction	%	37	28	14	0.22
Mortality	%	12.5	15.1	7.1	0.73
PaO_2 / FiO_2 (mm Hg)	Mean ± SD	407 ± 109	424 ± 68	353 ± 82	0.22
Oxygenation Index (cm H_2O/mm Hg)	Mean ± SD	1.5 ± 0.7	1.7 ± 0.5	1.7 ± 0.8	0.16
$PaCO_2$ (mm Hg)	Mean ± SD	37.0 ± 7.7	37.6 ± 10.0	39.2 ± 10.6	0.74
pH	Mean ± SD	7.36 ± 0.07	7.34 ± 0.12	7.37 ± 0.10	0.78
Blood product transfusion	Transfusions / pt	2.2	3.7	12.4	0.22
PEEP (cm H_2O)	Mean ± SD	4.7 ± 2.0	5.3 ± 2.7	3.6 ± 2.4	0.08
PIP (cm H_2O)	Mean ± SD	19.6 ± 7.1	19.9 ± 9.9	19.2 ± 6.2	0.97
MAP (cm H_2O)	Mean ± SD	12.1 ± 4.3	12.6 ± 5.5	11.5 ± 4.1	0.74
Vt (ml/kg)	Mean ± SD	8.2 ± 0.6	10.1 ± 0.7	14.2 ± 2.5	< 0.01

Table 2. *Admission characteristics of patients stratified for OR at end of observation*

		< 300 mm Hg	**≥ 300 mm Hg**	***p*-value**
Sex M/F	(%)	29/71	63/37	0.08
Age (years)	Median [range]	0.48 [0.1 - 8.3]	3.2 [0.1 - 13.7]	0.11
PRISM score	Mean ± SD	19.4 ± 4.9	16,3 ± 6.48	0.23
PIM score	Mean ± SD	2.36 ± 1.58	2.36 ± 1.80	0.99
Admission diagnosis				
Respiratory tract disease	(%)	28.6	7.7	0.86
Sepsis	(%)	0	25.0	0.13
Post-surgical	(%)	38	43	0.82
Neurologic dysfunction	(%)	0	26.9	0.11
Mortality	(%)	14.3	17.3	0.84
PaO_2/FiO_2 (mm Hg)	Mean ± SD	367.1 ± 60.6	434.8 ± 181.7	0.34
Oxygenation Index (cm H_2O/mm Hg)	Mean ± SD	2.24 ± 1.1	1.66 ± 0.76	0.08
$PaCO_2$ (mm Hg)	Mean ± SD	41.6 ± 10.8	38. ± 9.3	0.39
pH	Mean ± SD	7.33 ± 0.09	7.36 ± 0.11	0.49
Blood product transfusion	transfusion / patient	5.7	4.8	0.74
PEEP (cm H_2O)	Mean ± SD	4.9 ± 2.5	5.0 ± 2.2	0.90
PIP (cm H_2O)	Mean ± SD	24.3 ± 8.6	20.1 ± 10.1	0.30
MAP (cm H_2O)	Mean ± SD	14.6 ± 5.1	12.5 ± 5.4	0.35
Vt (ml/kg)	Mean ± SD	12.7 ± 4.8	9.9 ± 1.8	<0.01

Tidal volume and development of OR < 300 mm Hg

Initial Vt in group I, II, III were 8.2 ± 0.6 ml/kg (n = 24), 10.1 ± 0.7 ml/kg (n = 58), and 14.2 ± 2.5 ml/kg (n = 14), respectively. PIM, PRISM scores, mortality, admission OR, $PaCO_2$, pH, ventilator settings (PEEP, PIP, Mean Airway Pressure) were not statistically different between groups. Of the other variables, only age was significantly different (Table I).

In ventilated children the use of higher tidal volumes on admission was associated with increased development of a OR < 300 mm Hg and this effect became more pronounced during the course of mechanical ventilation (Figure 3a). Similar results were found for average Vt during MV (Figure 3b).

Tidal Volume in patients with OR < and > 300 mm Hg

At the end of observation period n = 40 (41%) of the 96 included children had developed OR < 300mm Hg. Neither PIM and PRISM scores nor admission OR were significantly different between groups, nor were age and other parameters (Table 2).

Development of OR < 300 mm Hg was associated with use of higher Vt. In children developing a OR < 300 mm Hg, Vt on admission had been 12.7 ± 4.8 ml/kg versus 9.3 ± 1.0 ml/kg in children with a OR > 300 mm Hg ($p = 0.05$). Similar differences were observed for average Vt during MV: 12,0 ± 3,5 ml/kg versus 8,7 ± 3,0 ml/kg ($p = 0.03$).

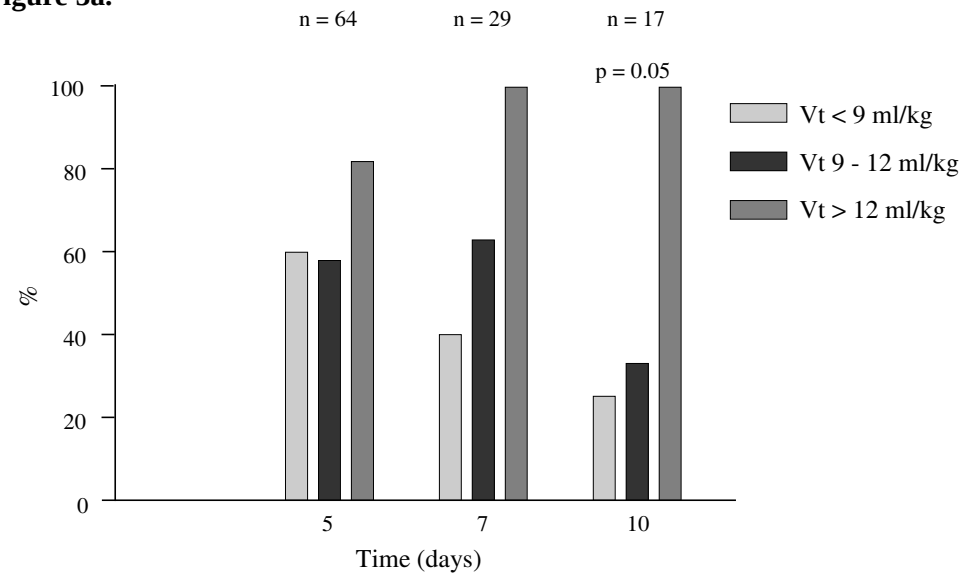
Positive End Expiratory Pressure and OR

Initial PEEP did not differ between groups I, II and III (Table 1) nor between patients that developed OR < 300 mm Hg and those who did not (Table 2). There was no significant increase over time between day 1 and 10 in PEEP in the whole cohort ($p = 0.44$), nor significant differences between groups I, II and III ($p = 0.69$) or between patients that developed OR < 300 mm Hg and those who did not ($p = 0.56$).

Tidal volume, Oxygenation Ratio and duration of MV

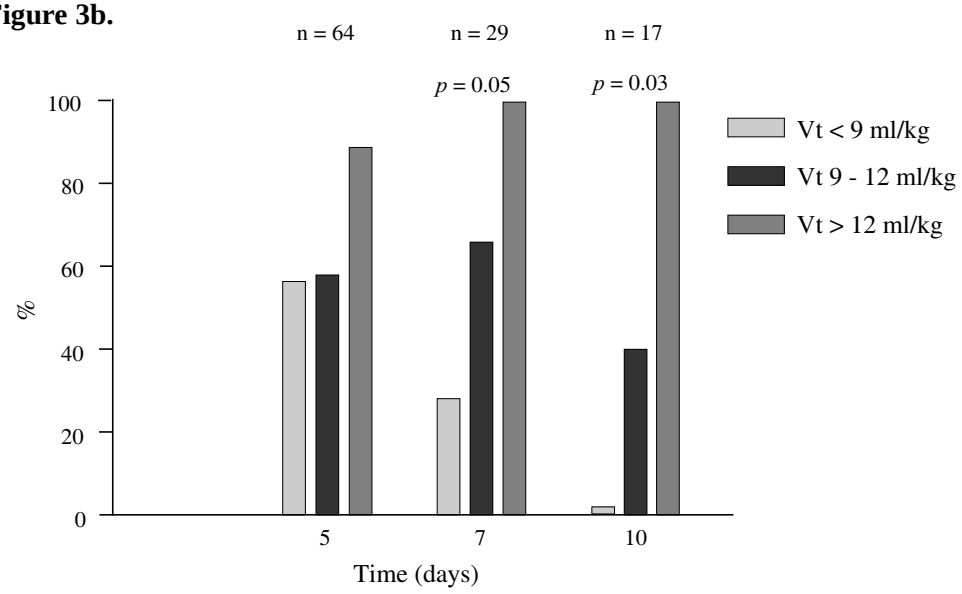
Percentages of patients being extubated did not differ between group I, II and III during the observation period ($p > 0.50$). However, development of an OR < 300 mm Hg was associated with longer MV duration. In children maintaining an OR > 300 mm Hg on day 5 median [range] MV duration was 7 [5 - 21] days versus 9 [5 - 54] days in children developing OR ratio < 300 mm Hg ($p = 0.06$) with similar but non-significant differences when developing an OR ratio < 300 mm Hg on day 7 and 10 ($p = 0.13$ and 0.20). For the total cohort median [range] was 5 [3 - 21] days in children in children maintaining OR > 300 mm Hg versus 9 [4 - 54] days in children developing OR < 300 mm Hg ($p < 0.01$).

Figure 3a.



Percentage of ventilated children developing PaO_2 / FiO_2 < 300 mm Hg, depending on initial tidal volume.

Figure 3b.



Percentage of ventilated children developing PaO_2 / FiO_2 < 300 mm Hg, depending on average tidal volume.

Discussion

The results from this study suggest that in ventilated critically ill children with an initial normal gas exchange, a Vt > 9 ml/kg increases the risk for VALI, which may result in a prolonged duration of mechanical ventilation. As the baseline PRISM / PIM scores and OR in the 3 groups were not significantly different, the observed relation between large Vt and the development of VALI cannot be explained by differences in admission disease severity. Our results are in concordance with a similar retro spective study performed in adult ICU patients with normal pulmonary function on admission. In that study a Vt > 6 ml/kg was a risk factor for the development of VALI with an odds ratio of 1.3 for each ml/kg above 6 ml/kg.[2] Mean Vt (10.9 ± 2.3 ml/kg) in their group was comparable with ours, but Vt distribution showed a larger percentage of patients receiving Vt > 12 ml/kg. However, the overall incidence of VALI was 24%, compared to 41% in our study. This could indicate that children are even more susceptible to the effects of high tidal volumes than ventilated adults. In addition to differences in physiology, small children have a lower lung / bodyweight ratio, hence a Vt based on bodyweight results in a larger Vt / lungweight ratio than in adults. Moreover, our observation of a mean Vt on day 1 of 10.2 ± 2.2 ml/kg, which is substantially higher than the Vt of 6 ml/kg generally considered lung-protective, indicates that a large proportion of children are still ventilated with tidal volumes that are too high. This observation is also in concordance with several studies in the adult population that have shown that actual applied Vt is usually higher than advocated.[20-22]

We observed that with longer periods of mechanical ventilation the difference in VALI increased between patients ventilated with small and large Vt. This may indicate that clinical lung injury induced by large Vt becomes apparent only after several days, suggesting a protracted injurious effect in which inflammation is probably involved. The inflammatory response to MV is thought to be the central mechanism in the pathogenesis of VALI.[23] In the ARDS Network trial it was demonstrated that patients ventilated with Vt > 12 ml/kg not only had an increased mortality rate, but also higher pro-inflammatory cytokine levels.[1] This is consistent with a study by Plotz et al. in which 12 children without lung injury, ventilated for cardiac catheterization (Vt 10 ml/kg, PEEP 4 cm H_2O, FiO_2 0.4) showed a marked pro-inflammatory response in alveolar lavage fluid.[24] The use of very small Vt however may also exert adverse effects such as loss of aeration and subsequent atelectrauma. A recent study on lung inflammation in neonates with IRDS revealed increased pulmonary inflammatory mediators and prolonged duration of mechanical ventilation when ventilated with Vt 3 ml/kg versus 5 ml/kg which is below the physiologic range of spontaneous Vt.[11,25,26] Over the recent years use of large Vt has decreased and possibly contributed to a

decrease in the development of ventilator associated lung injury, as suggested in a retrospective study.[27] However, no prospective mechanical ventilation studies in children are available, and the optimal Vt in children awaits to be established.

PEEP is another important variable possibly associated with VALI, but in our study PEEP was not significantly different between patients developing OR < 300 and those who did not. As PEEP level during later stages of MV may have been adjusted to the OR and therefore is not an independent variable, we only analyzed the influence of initial PEEP levels on the development of OR < 300 mm Hg. Our finding that the initial PEEP level was not associated with subsequent development of OR < 300 mm Hg is consistent with the study of Gajic et al.[2] In adult patients with established lung injury, the largest currently available prospective study did not find an effect of different PEEP levels in patient outcome either.[28] The applied PEEP level in our study of 4.9 ± 2.5 cm H_2O is consistent with the PEEP level necessary to prevent atelectasis in healthy children during anesthesia.[29]

Several limitations of the present study should be discussed. First, the relatively small number of patients and the retrospective study design preclude any firm conclusion, and the putative protective effects of smaller Vt should be confirmed by a prospective study, preferably focusing on a Vt below 9 ml/kg. As in our data set minimal applied Vt was 6.8 ml/kg, and only 22.9% of children were ventilated with a Vt < 9 ml/kg, subdivision of Vt in the range below 9 ml/kg resulted in groups too small for analysis.

Also, heterogeneity is observed regarding the age of included children. Although a statistical difference in baseline age between group I, II and III was found, no significant difference in age was observed between patients that developed OR < 300 mm Hg compared to those who did not. These findings suggest that younger children are more often ventilated with large tidal volumes, but that age by itself is not a risk factor for the development of OR < 300 mm Hg. We also analyzed data of subgroups of patients below 10 years and below 6 years of age. These reveal similar results, however as group size diminishes, statistical analysis becomes weaker (Type I error), so we chose to present the whole cohort. Possibly less consideration was given to the size of Vt in the youngest group of patients, probably due to rounding off of Vt or accepting differences between prescribed and delivered Vt. Most ventilators measure Vt within the ventilator itself and this technical inaccuracy is more important in the smaller infant. However, no reliable correction can be made for compressible volume and tidal volume reading is the only bedside available information for the clinician guiding the ventilator settings.

The fact that patients that were diagnosed with VAP during mechanical ventilation

were excluded may introduce a confounder, however they were equally distributed between groups. In addition, the observed prevalence of VAP is within the range reported in adults.[30]

Conclusion

Our study shows that in children with a normal OR on ICU admission, ventilation with a Vt > 9 ml/kg is associated with the development of VALI. Hence, although optimal Vt is unclear in ventilated children, children are likely to benefit from a lung protective ventilation strategy similar as observed in adult ICU patients. As pediatric physiology is essentially different from adults, results from adult ICU studies should be evaluated in the pediatric critical care population.

Abbreviations

MV	mechanical ventilation
ICU	Intensive Care Unit
Vt	tidal volume
PEEP	positive end expiratory pressure
VALI	ventilated associated lung injury
ALI	acute lung injury
OR	oxygenation ratio
PICU	Pediatric Intensive Care
PIM	pediatric index of mortality
PRISM	pediatric risk of mortality
VAP	ventilator associated pneumonia
TRALI	tranfusion related avute lung injury
IRDS	infantile respiratory distress syndrome

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Chapter 9

Summary and General Discussion / Samenvatting

Summary

In this thesis we investigated the relation between mechanical ventilation, inflammation and lung injury. The thesis is divided into an experimental and a clinical part. In the experimental part, we developed an animal model to study the role of stretch of the cell membrane (*mechanotransduction*) during low tidal volume ventilation in *Ventilator Induced Lung Injury* (VILI). In the clinical part, we investigated the role of tidal volume and recruitment maneuvers on VILI in critically ill children.

Part I experimental studies

Chapter 2 is a review on the role of inflammation and more specifically cytokines and leukocytes in VILI. Two processes appear to be involved in mechanical ventilation-induced cytokine increase. *In-vitro* stretching of pulmonary and non-pulmonary cells results in increased synthesis of both pro- and anti-inflammatory cytokines, a process called *mechanotransduction*. *Ex-vivo* and *in-vivo* studies demonstrate that the loss of cellular plasma membrane integrity resulting from the use of high tidal volumes or shear stress phenomena (baro-volutrauma) results in *decompartimentalization* with an increase in cytokine levels in the alveolar space and the systemic circulation. These two mechanisms are highly interrelated: baro-volutrauma evokes biotrauma, and biotrauma aggravates baro-volutrauma as well. Ventilator settings, especially tidal volume, positive end expiratory pressure (PEEP) level and lung recruitment maneuvers appear to affect cytokine levels in VILI . The role of cytokines in the pathogenesis of VILI has been shown in multiple experimental studies using specific anti-cytokine antibodies or receptor blockers which reduce the amount of lung damage in several models. Clinical studies in patients with Adult Respiratory Distress Syndrome (ARDS) show that non-survivors have an increased and persistent pro-inflammatory response with increased cytokine levels in both the alveolar space and the systemic circulation.

Leukocyte influx is a hallmark of VILI , and propagates pulmonary cytokine up-regulation, however the role of leukocytes is not entirely clear. Obviously, VILI is attenuated in experiments with induced leucopenia but other resident cells (alveolar macrophages and type II alveolar epithelial cells) also contribute to lung injury.

In *Chapter 3* we describe a new model to study the role of mechanotransduction in VILI . We ventilated healthy mice with a so-called *Lung Protective Ventilation Strategy* (LPVS) with moderate tidal volumes of 8 ml/kg, PEEP of 4 cm H_2O and a FiO_2 0.4. We showed that this form of mechanical ventilation triggered an inflammatory response without evoking histological or cellular damage (*mechanotransduction*). The response starts with an elevation of keratinocyte derived chemokine (KC) after 30 minutes,

followed by leukocyte influx and up-regulation of other cytokines such as interleukin (IL)-1α, IL-1β, Tumor Necrosis Factor (TNF)-α, IL-6 and IL-10. Animals allowed to recover for 3 days after a period of 4 hours of mechanical ventilation showed normal cytokine levels and no leukocyte influx. Mechanical ventilation in leukocyte-depleted animals showed less but not absent pulmonary cytokine release, suggesting that besides leukocytes also other cells (for example Type-II alveolar cells) play a role in cytokine up-regulation.

Chapter 4 describes the effect of $PaCO_2$ on the mechanical ventilation induced pulmonary cytokine release and leukocyte influx. Lung protective ventilation strategy, using small tidal volumes, frequently results in higher $PaCO_2$, called "*permissive hypercapnia*". Clinical studies applying LPVS resulting in higher $PaCO_2$ levels show a decrease in VILI . It is unclear whether the lower tidal volume itself or the subsequent higher $PaCO_2$ concentrations account for the observed protective effects.

In this study we used the previously described mouse model with identical ventilator settings but increased the $PaCO_2$ by adding CO_2 to inhaled gas. Hypercapnic mechanical ventilation significantly attenuated the mechanical ventilation induced IL-1β, TNF-α, IL-6, IL-10 and KC increase with 16 - 25% and 45 - 89% with $FiCO_2$ 2% and 4%, respectively. Quantitative microscopic analysis showed a 4.7 ± 3.7 fold increase in pulmonary leukocyte influx in normocapnic ventilated animals and a significant reduction in leukocyte influx of 57 ± 32% and 67 ± 22% when ventilated with $FiCO_2$ 2% and 4%, respectively.

These data demonstrate that hypercapnic acidosis has an independent, additional effect on pulmonary cytokine release and leukocyte influx that may explain part of the protective effect of the use of small tidal volumes on VILI during *permissive hypercapnia*. Further studies should demonstrate whether *permissive hypercapnia* is therapeutic and should be incorporated in Lung Protective Ventilations Strategy.

Part II clinical studies in critically ill children

Chapter 5 reviews the effects of recruitment maneuvers during conventional mechanical ventilation. Recruitment maneuvers are performed to revert lung collapse resulting from low tidal volume ventilation, and improve gas exchange. Experimental studies clearly show beneficial effects of recruitment after surfactant dysfunction and provide insight in the role of the Peak Inspiratory Pressure (PIP) and PEEP as expressed by the amount of lung aeration and gas exchange. However, experimental studies also show that recruitment maneuvers may translocate inflammatory mediators and bacteria from the alveolar space into the systemic circulation.

Clinical studies show divergent results. In the healthy lung (e.g. during anesthesia)

recruitment maneuvers increase oxygenation, but in the diseased lung in ICU patients recruitment maneuvers rarely result in a long lasting significant increase in oxygenation often accompanied by adverse hemodynamic effects. Recruitment maneuvers using high inflation pressures and performed early in the disease process result in the largest improvement in oxygenation. Recently, the ALVEOLI trial and LOVS trial reported serious side effects of recruitment procedures in adults with ARDS. An improvement in outcome has not been shown. In infants the effect of recruitment maneuvers on gas-exchange and inflammatory response has not been studied.

Chapter 6 describes and analyzes the results of a national survey on the use of recruitment maneuvers in all 8 Pediatric Intensive Care Units (PICUs) and 10 Neonatal Intensive Care Units (NICUs) in the Netherlands. Despite the lack of clinical proof of their beneficial effects, all PICUs and 85% of NICUs in the Netherlands use recruitment maneuvers. Both manual ("bagging") (*PICU 100%, NICU 85%*) and ventilator assisted recruitment maneuvers (e.g. sustained inflation, "opening procedures") (*PICU 100%, NICU 57%*) are used. When ventilator-assisted recruitment maneuvers are used, they mostly consist of combined PEEP and PIP elevation. Interestingly, most PICUs used distinctively higher pressures than were used in NICUs when using mechanical recruitment procedures: PEEP 28.3 ± 7.5 versus 9.2 ± 1.1 H_2O respectively, PIP 46.7 ± 12.1 versus 35.8 ± 4.9 cm H_2O. Several adverse effects of recruitment maneuvers are reported, predominantly affecting hemodynamic stability. These results indicate the need for randomized controlled trials investigating the assumed beneficial effects and safety of recruitment maneuvers.

In *Chapter 7* we studied the effects of a single combined PEEP / PIP elevation recruitment maneuver on gas exchange and cytokine levels in tracheo-bronchial lavage fluid and plasma in children with acute lung injury. We noted a marked, rapid and transient increase in plasma TNF-α, IL-1β and IL-6 levels 15 minutes after recruitment, indicating translocation from the alveolar space into the circulation. Also, the tracheo-bronchial lavage fluid showed a trend towards increased IL-8 levels at the end of the 360 minutes observation period, potentially indicating an augmented alveolar inflammatory response. Furthermore we observed no increase in oxygenation nor the need for lower ventilatory pressures after the recruitment maneuver. This study demonstrates that in contrast to two studies using short-term recruitment maneuvers in adults, a more extensive recruitment maneuver in children may increase systemic cytokine levels, and should not be used routinely.

Chapter 8 is a retrospective analysis of the effect of tidal volume and PEEP on the development of *Ventilator Induced Lung Injury* in children without acute lung injury as indicated by an initial PaO_2 / FiO_2 ratio > 300 mm Hg. We show that in our cohort, children initially ventilated with a higher tidal volume, demonstrate increasing rates of lung dysfunction defined as PaO_2 / FiO_2 ratio < 300 mm Hg. Furthermore, the observed differences increased with duration of ventilation. Development of a PaO_2 / FiO_2 ratio < 300 mm Hg was associated with longer duration of mechanical ventilation. This study demonstrates that even children with normal gas exchange should be ventilated with tidal volumes below 9 ml/kg.

General discussion

Future tools in reducing *Ventilator Induced Lung Injury*

Understanding the pathophysiology of *Ventilator Induced Lung Injury* is essential to initiate further clinical studies that may provide us with new essential information to improve the currently accepted *Lung Protective Ventilation Strategy* (LPVS).

The experimental studies performed in this thesis give further insight in the pathophysiology of *Ventilator Induced Lung Injury*, and how these experimental observations can be translated into clinical practice in ventilated critically ill children. Previously, cytokine up-regulation by mechanical ventilation has been demonstrated in experimental and clinical studies using either injurious high tidal volumes in healthy lungs or low tidal volumes in pre-injured lungs. Our observation that even so-called LPVS, without damage to the pulmonary ultra-structure, results in up-regulation of pulmonary cytokines and leukocyte infiltration with translocation into the systemic circulation is pathophysiologic important. It demonstrates that mechanotransduction-induced inflammation may precede histological lung injury and the immunologic response potentially makes the lung more vulnerable to a second insult and may contribute to Multi Organ Dysfunction Syndrome.

In this mechanotransduction model, current and future studies by our group using kock-out animals enable us to analyze the intra- and extra-cellular inflammatory ediators involved in the innate immune response. Furthermore, several other important pathophysiologic mechanisms can be explored such as gene up-regulation and expression (e.g. activation of inflammatory or apoptotic genes in cells involved in *Ventilator Induced Lung Injury*); cell surface antigen / receptor expression (e.g.

Intracellular Adhesion Molecule, Vascular Cell Adhesion Molecule, P-selectin involved in leukocyte adherence and influx); or changes in cellular components (e.g. cell membrane and matrix) and growth factors (e.g. Endothelial Growth Factor, Transforming Growth Factor-α, β) involved in fibroproliferation and resolution during various ARDS stages. This might lead to new therapeutic tools to reduce lung injury, by selective blocking of mediators induced by mechanotransduction only, leaving the inflammatory response to other signals intact.

This experimental model also allows us to study non-selective modulating effects of the inflammatory response evoked by mechanical ventilation. Very interesting is the protective effect of "therapeutic" hypercapnia on pulmonary cytokine release and leukocyte influx, although much more work needs to be done. The conducted *in-vivo* study combines observations from both *in-vitro* and *ex-vivo* experiments in isolated perfused lungs. The effect of CO_2 appears to be mediated by the pH as buffering of the respiratory acidosis attenuates the effect. The exact pathways by which CO_2 decreases this inflammatory response needs further study. It is not known whether the observed beneficial effect of hypercapnia *in-vivo* also exist in this model when combined with a second hit (e.g. pre-existing lung injury, endotoxin challenge).

Although the attenuating effect of hypercapnic acidosis on inflammatory response appears dose-related, the optimal pulmonary protective CO_2 level *in-vivo* needs to be determined, as the cumulative effect of hypercapnic acidosis on other protective mechanisms, both biochemical (e.g. xanthine oxidase inhibition, oxygen radical formation, decreased complement activation, delayed apoptosis) or regulatory (e.g. ventilation/perfusion match, pulmonary vascular resistance, oxygen dissociation curve shift) is not known. To investigate the net effect of hypercapnia on both lung-injury and extra-pulmonary organs, e.g. cerebral perfusion, and myocardial function, prospective clinical studies are needed. Especially in newborn infants with immature auto-regulation of cerebral blood flow, clinical studies should demonstrate whether and what level of hypercapnia improves outcome.

Given the fact that the experimental studies have shown that positive pressure ventilation evokes a pulmonary inflammatory response preceding lung injury and gas exchange disorders, clinical trials evaluating tools of lung protective strategies should primarily focus on minimizing this response and the resulting histological damage and less on improving short-term variables such as oxygenation ratio or lung mechanics. For instance the ARDS network trial demonstrated that ventilation with a tidal volume of 12 ml/kg initially resulted in a higher PaO_2 / FiO_2 ratio but eventually resulted in higher IL-6 levels and a higher mortality. Apparently, large tidal volumes initially

increase lung recruitment resulting in improved oxygenation, but this does not outweigh the long-term injurious effects of increasing inflammation.

In critically ill children however, a similar prospective study is lacking and in current practice "optimal tidal volume" and other tools, are mainly used by copying results obtained in adult patients, despite essential differences in anatomy and physiology. In our retrospective cohort of critically ill children we found that a tidal volume over 9 ml/kg is associated with an increased chance of developing lung injury. As the prevalence of lung injury increased over time, an ongoing injurious trigger is likely. We assume that this trigger is persistent inflammation which, also in children, eventually contributes to deteriorating gas exchange and lung injury.

Furthermore, our observation that even a single recruitment maneuver with a short lasting increase in ventilatory pressures - with the aim of increasing oxygenation - translocates inflammatory mediators into the circulation supports this as well.

In the clinical search for additional tools to reduce inflammation and lung injury, we need to focus on ventilator setting variables other than tidal volume, and in resetting our goals of gas exchange.

One of the potential new tools is the ventilator mode, especially spontaneous or assisted ventilation in contrast to controlled ventilation. Experimental studies have already shown that spontaneous ventilation during mechanical ventilation reduces *Ventilator Induced Lung Injury* compared with paralyzed subjects. No data on the effect of ventilation mode on lung injury in children are known, but in a cohort of adults admitted to our ICU with normal PaO_2 / FiO_2 ratio and ventilated with a Lung Protective Ventilation Strategy (tidal volume 7.9 ± 0.1 ml/kg, peak pressure 19.5 ± 4.1 cm H_2O; PEEP 5.9 ± 1.9 cm H_2O) we identified pressure control ventilation mode as a significant, independent factor to develop a PaO_2 / FiO_2 ratio below 300 mm Hg, compared with pressure support mode (Odds Ratio 4.3). Also other studies suggest that modes using spontaneous breathing during mechanical ventilation such as Airway Pressure Release Ventilation (APRV) may reduce lung injury. This illustrates that the choice of ventilatory assist mode, may become another important bedside tool of the Lung Protective Ventilation Strategy. Also, new techniques improving patient-ventilator interaction such as automated maltriggering detection or Neurally Adjusted Ventilatory Assist may further contribute to decreasing *Ventilator Induced Lung Injury* by allowing earlier spontaneous ventilation.

Non-invasive mechanical ventilation modes (NIMV) such as nasal Continuous Positive Airway Pressure or nasal Intermittent Mandatory Ventilation, is well tolerated and feasible and effective in supporting respiration in small infants and neonates. In specific conditions, it appears to avoid or shorten endotracheal intubation and ventilation, and respiratory support with NIMV is probably less injurious. However, in

the larger infant and child feasibility is still a problem that precludes its clinical use in this population.

Other future directions to explore should focus on resetting the goals of gas exchange during mechanical ventilation. Not only permissive hyper*capnia*, but also permissive hyp*oxemia* may become an important tool, especially in the newborn infant. In the neonate the transition of fetal to extra-uterine life implies an increase in PaO_2 from ≤ 40 mm Hg to > 70 mm Hg and $TcSaO_2$ 60% increasing to > 97%. In many neonatal ICUs targeted transcutaneous SaO_2 is between 88% and 95%. Aiming at lower $TcSaO_2$ during mechanical ventilation in the initial phase of extra-uterine life may reduce the risk of lung injury, e.g. through diminished oxygen toxicity as suggested in experimental studies, and through reduced ventilatory pressures and subsequent inflammation. However, similar as with permissive hypercapnia, prior to clinical implementation further experimental studies and clinical randomized controlled trials on the pulmonary inflammatory response, the effects on the lung and the other organs, especially the brain and neurodevelopment, are needed.

Although mechanical ventilation continues to be one of the most important lifesaving tools in the pediatric and neonatal intensive care unit, its adverse effects necessitate ongoing study to improve Lung Protective Ventilation Strategy. The results of experimental studies, e.g. as in this thesis, should guide clinical randomized controlled trials needed before implementation of these strategies in clinical practice. For the pediatric and neonatal intensive care unit, translation of both experimental and clinical ICU evidence into the specific pediatric critically ill population is mandatory prior to clinical use of new lung protective tools.

Samenvatting

In dit proefschrift hebben wij de relatie onderzocht tussen mechanische ventilatie, inflammatie en long schade. Het proefschrift is verdeeld in een experimenteel deel en klinisch deel. Voor het experimentele deel ontwikkelden wij een model om de rol van cyclische rek van de celmembraan in *Ventilator Induced Lung Injury* (VILI) tijdens beademing met lage teugvolumes te bestuderen (*mechanotransductie*). In het klinische deel, onderzochten wij de rol van teugvolumes en de recruteer manoeuvres op VILI in kritisch zieke kinderen.

Deel I: experimentele studies

Hoofdstuk 2 is een overzicht van inflammatie, en meer specifiek de rol cytokines en leukocyten in *Ventilator Induced Lung Injury*. Twee processen blijken te worden geïnduceerd bij door mechanische ventilatie veroorzaakte cytokine *up-regulation*. Uit *in-vitro* onderzoek blijkt dat rek van zowel pulmonale als niet-pulmonale cellen resulteert in verhoogde concentraties van zowel pro- als anti-inflammatoire cytokines: *mechanotransduction. Ex-* en *in-vivo* experimenten tonen aan dat het verloren gaan van de cellulaire integriteit door schade aan de plasmamembraan als gevolg van het gebruik van o.a. hoge teugvolumes (baro-volutrauma) in resulteert *decompartimentalisatie*: verspreiding van cytokines naar de alveolare ruimte en de systemische circulatie. Deze twee mechanismen zijn nauw met elkaar gerelateerd: baro-volutrauma induceert biotrauma, en biotrauma verergert de gevoeligheid voor baro-volutrauma. De instelling van het beademingsapparaat, vooral teugvolume, *Positive End Expiratory Pressure* (PEEP), en long recruteer manoeuvres blijken cytokine niveaus in VILI te beïnvloeden. De rol van cytokines in de pathogenese van VILI blijkt uit verscheidene experimentele studies, waarin gebruik van specifieke anti-cytokine antilichamen of receptor-blokkers de mate van longschade vermindert. Klinische studies bij patiënten met *Adult Respiratory Distress Syndrome* (ARDS) tonen aan dat bij non-survivors ten op zichte van survivors een verhoogde en blijvende pro-inflammatoire ontstekingsreactie hebben met verhoogde cytokine concentraties in zowel de alveolare ruimte als de systemische circulatie.

De influx van leukocyten is een van de pathofysiologische kenmerken van VILI, en draagt verder bij aan de cytokine *up-regulation*, hoewel nog niet alle aspecten volledig opgehelderd zijn. In experimenten met leukopene dieren blijkt VILI sterk verminderd, echter ook andere cellen zoals alveolare macrofagen en type II alveolare epitheel cellen blijken bij te dragen aan beademings gerelateerde longschade.

In *Hoofdstuk 3* beschrijven wij een nieuw experimenteel model om de rol van mechanotransduction in VILI te bestuderen. Gezonde muizen werden beademd met zogenaamde *Lung Protective Ventilation Stategy* (LPVS): kleine teugvolumes van 8 ml/kg, PEEP van 4 cm H_2O en een FiO_2 0.4. Wij toonden aan dat deze vorm van mechanische ventilatie een ontstekingsreactie teweegbracht zónder histologische of cellulaire schade te induceren (*mechanotransduction*). De reactie begint met een verhoging van *keratinocyte derived chemokine* (KC) na 30 minuten, welke door leukocyten influx en *up-regulation* van andere cytokines, zoals interleukin (IL)-1α, IL-1β, TNF-α, IL-6 en IL-10 wordt gevolgd. Drie dagen na een periode van 4 uur mechanische ventilatie werden genormaliseerde cytokine en leukocyten niveaus waargenomen. Mechanische ventilatie in leukocyt-deplete dieren toonde minder, doch niet geheel afwezige pulmonale cytokine stijging, hetgeen aantoont dat naast leukocyten ook andere cellen (bijvoorbeeld type II alveolare cellen) een rol spelen bij cytokine *up-regulation*.

Hoofdstuk 4 beschrijft het effect van de $PaCO_2$ op de door mechanische ventilatie veroorzaakte leukocyten influx en cytokine *up-regulation*. Het gebruik van kleine teugvolumes tijdens *Lung Protective Ventilation Strategy* resulteert vaak in hogere $PaCO_2$ concentraties, genaamd "permissive hyparcapnia". Klinische studies die het teugvolume verminderen en de piekdruk beperken, hetgeen in hogere $PaCO_2$ kan resulteren, tonen een vermindering van de mate van *Ventilator Induced Lung Injury*. Het is onduidelijk of de lagere teugvolumes zelf, of de hieruit voortvloeiende hogere $PaCO_2$ concentraties verantwoordelijk zijn voor de protectieve gevolgen.

In deze studie gebruikten wij het eerder beschreven experimentele model, maar verhoogden de $PaCO_2$ door CO_2 toe te voegen aan het geïnhaleerde gas bij een identieke beademings instelling. Hypercapnische ventilatie toonde een significante vermindering van de door mechanische ventilatie veroorzaakte stijging van IL-1β, TNF-α, IL-6, IL-10 en KC met 16 - 25% en 45 - 89% bij $FiCO_2$ 2% en 4%, respectievelijk. De kwantitatieve microscopische analyse toonde een verhoging van pulmonale leukocyten influx met een factor 4.7 ± 3.7 in normocapnisch geventileerde dieren, en een significante vermindering van leukocyten influx na hypercapnische beademing met 57 ± 32% en 67 ± 22% bij respectievelijk $FiCO_2$ 2% en 4%.

Deze data tonen aan dat hypercapnische acidose een onafhankelijk, additioneel effect op de pulmonale cytokinestijging en leukocyten influx heeft, hetgeen een deel van het beschermende effect van het gebruik van kleine teugvolumes bij VILI tijdens *permissive hypercapnia* kan verklaren. Verdere studies zullen moeten aantonen of *permissive hypercapnia* therapeutisch is en in *Lung Protective Ventilation Stategy* dient te worden geïncorporeerd.

Deel II klinische studies in kritisch zieke kinderen

Hoofdstuk 5 geeft een overzicht van de resultaten van recruteer manoeuvres tijdens conventionele mechanische ventilatie. Deze recruteer manoeuvres worden uitgevoerd om alveolaire collaps als gevolg van ventilatie met kleine teug volumes te herstellen, en daarmee de gasuitwisseling te verbeteren.

Experimentele studies tonen duidelijk gunstige effecten van recruteer manoeuvres bij surfactant deficiëntie / dysfunctie, en verschaffen inzicht in het effect van de Peak Inspiratory Pressure (PIP) en Positive End Expiratory Pressure (PEEP) op longcollaps en gaswisseling tijdens recruteren. Echter, experimentele studies tonen ook aan dat de recruteer manoeuvres ontstekingsmediatoren en bacteriën van de alveolare ruimte naar de systemische circulatie kunnen transloceren.

Klinische studies daarentegen tonen uiteenlopende resultaten. In de gezonde long, bijvoorbeeld tijdens anesthesie, verbeteren recruteer manoeuvres de oxygenatie, terwijl in de zieke long in IC patiënten ICU recruteer manoeuvres zelden tot een langdurig significante verbetering van oxygenatie leiden, en vaak ongunstige hemodynamische gevolgen hebben. Recruteer manoeuvres welke vroeg in het ziekteproces toegepast worden lijken de grootste verbetering van oxygenatie te bewerkstelligen. Recent gepubliceerde ALVEOLI en OLVS trials tonen ernstige bijwerkingen van recruteer manoeuvres in volwassenen met ARDS. Een overtuigende verbetering van de uitkomst in deze populatie is tot nog toe niet aangetoond.

In kritisch zieke kinderen is het effect van recruteer manoeuvres op de gas-uitwisseling en de ontstekingsreactie nog niet goed bestudeerd.

Hoofdstuk 6 beschrijft en analyseert de resultaten van een nationaal onderzoek naar het gebruik van recruteer maneuvers in alle 8 Pediatrische Intensive Care Units (PICUs) en 10 Neonatale Intensive Care Units (NICUs) in Nederland. Ondanks het gebrek aan klinisch bewijs van de gunstige effcten, gebruiken alle PICUs en 85% van de NICUs van Nederland recruteer manoeuvres. Zowel handmatige ("balloneren") (*PICU 100%, NICU 85%*) als ventilator-gestuurde recruteer manoeuvres (b.v. *sustained inflation, opening procedures*) (*PICU 100%, NICU 57%*) worden gebruikt. Wanneer de ventilator-gestuurde recruteer manoeuvres worden gebruikt, bestaan zij meestal uit de gecombineerde verhoging van het PEEP en PIP. Opvallend is dat de meeste PICUs significant hogere drukken gebruiken dan de NICUs bij het toepassen van recruteer manoeuvres: PEEP 28.3 ± 7.5 versus 9.2 ± 1.1 H_2O respectievelijk PIP 46.7 ± 12.1 versus 35.8 ± 4.9 cm H_2O. Verscheidene ongunstige gevolgen van rekruterings-manoeuvres werden gerapporteerd, hoofdzakelijk met betrekking tot hemodynamische stabiliteit. Deze resultaten benadrukken de noodzaak van *randomized controlled trials* om de gevolgen en de veiligheid van recruteer manoeuvres vast te stellen.

In *Hoofdstuk 7* bestudeerden wij de gevolgen van één enkele recruteer manoeuvre, bestaande uit gecombineerde verhoging van PEEP en PIP, op de gas uitwisseling en cytokine concentraties in tracheo-bronchiale lavage vloeistof en plasma in beademde kinderen met *Acute Lung Injury*. Er werd een duidelijke, snelle maar voorbijgaande verhoging van plasma TNF-α, IL-1β en IL-6 concentratie 15 minuten na recruteren geobserveerd, hetgeen wijst op translocatie vanuit de alveolare ruimte naar de circulatie. Tevens toonde analyse van de tracheo-bronchiale lavagevloeistof een tendens naar verhoogde IL-8 concentraties aan tegen het eind van de 360 minuten durende observatie periode, mogelijk wijzend op een toegenomen alveolare ontstekingsreactie. Er werd géén verbetering van de oxygenatie, noch lagere beademingsdrukken na het recruteer manoeuvre geobserveerd.

Deze studie toont aan dat, in tegenstelling tot twee studies waarin kortdurende recruteer manoeuvres bij volwassenen werden toegepast, een uitgebreidere recruteer manoeuvre in kritisch zieke kinderen de systemische cytokine concentraties wel kan verhogen, en derhalve niet routinematig zou moeten worden gebruikt.

Hoofdstuk 8 is een retrospectieve analyse van het effect van teugvolume en PEEP op de ontwikkeling van *Ventilator Induced Lung Injury* in kritisch zieke kinderen zonder *Acute Lung Injury*. Wij tonen aan dat kinderen met een opname PaO_2 / FiO_2 ratio > 300 mm Hg, welke aanvankelijk met een hoger teugvolume beademd worden in toenemende mate gaswisseling dysfunctie vertonen, gedefiniëerd als PaO_2 / FiO_2 ratio < 300 mm Hg. De geobserveerde verschillen namen met duur van de beademing toe. Het ontwikkelen van een PaO_2 / FiO_2 < 300 mm Hg was geassocieerd met een langere beademingsduur.

Deze studie toont aan dat ook kinderen met een initieel normale gas wisseling moeten worden beademd met teugvolumes kleiner dan 9 ml/kg.

Chapter 10

Dankwoord

Dankwoord

Dat dit proefschrift is tot stand gekomen naast alle andere zaken is mede te danken aan een aantal enthousiaste mensen, niet alleen diegenen die het werk hebben ondersteund, maar ook diegenen met goede ideeën of een kritisch oor, en die “het zin in onderzoek doen als alles lijkt tegen te zitten” weer op de voorgrond wisten te zetten.

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binnenkort ook staat met een mooi boekje!

Mr. dr. Erik-Jan Wervelman, paranimf. Beste Wervel, dank voor je regelematige telefoontjes (de promotiepolitie). Dat je door je eigen onderzoek ervaring weet hoe je de voortgang bewaakt en stimuleert werd zeer gewaardeerd. Dank dat je me bijstaat!

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Peter Andriessen, Sidarto Bambang Oetomo, Vinus Bok, Jan Buijs, Koen Dijkman, Carola Duister, Rene van Gent, Luc Janssen, Martin de Kleine, Thilo Mohns, Chris Schroër, Jacqueline van der Sluijs, Walther Tjon A Ten, Marjo Verreussel, Diana Visser, Janneke van Wijk, Bas Zegers, kinderartsen in Máxima Medisch Centrum in Veldhoven. Het is belangrijk dat je je gewaardeerd en thuis voelt op je werk tussen je collega's, en dat is absoluut wat ik bij jullie ervaar. Ik denk dat we een bijzondere vakgroep zijn met een fantastische toekomst! Dank voor de ruimte om nieuwe initiatieven te ontplooien zoals sedatie-unit, Level 1 IC / HC, ons landelijk ROP- en regionaal chirurgisch centrum. Maar ook voor het begrip en de clementie dat ik af en toe door "het boekje" werd opgeslokt, anders was het niet mogelijk geweest het boekje te schrijven. Wees gerust het was eens maar nooit weer….

Natuurlijk maken ook de arts-assistenten en nurse-practitioners, verpleging en

secretaresses deel uit van ons mooie Máxima Kinder team!

Laji Varghese, Wim van Dorp jullie zijn twee artsen van wie ik veel geleerd heb en waar ik veel bewondering voor heb hoe jullie als mens met je beroep omgaan. Dat zijn ook essentiële kwaliteiten om tijdens je vorming als arts te leren. Dank daar voor.

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Lieve kinderen Ruben, Jelske en Matthijs: Sinterklaasgedichten maken en in vakantiedagboeken tekenen is véél leuker dan artikelen schrijven en figuren maken! Gelukkig komt daar nu meer tijd voor!

Liefste Menne, wat heerlijk dat jij mijn life-line was in deze tijd van dead-lines! Heerlijk om met jou door het leven te mogen gaan en lief en leed te delen!

Chapter 11

List of Publications

Curriculum Vitae

1. M.A. Vos, A.P. Gorgels, T. Nagel, F.J.J. Halbertsma, H. Wellens: Further observations to confirm the arrhythmia mechanism-specific effect of flunarizine.
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Curriculum Vitae

De auteur werd geboren te Doorn op 4 april 1967. Na het eindexamen VWO aan de Vrije School te Zeist in 1986 studeerde hij geneeskunde aan de Rijks Universiteit Limburg, en Violoncello aan het Maastrichts Conservatorium bij Prof. Alexander Petrasch. In 1991 werd het doctoraal examen geneeskunde werd behaald; in 1992 werd het solo-diploma Uitvoerend Musicus Violoncello behaald. In het voorjaar van 1995 werd het arts-examen behaald aan het AZVU.

Geïnspireerd door de werkervaring van zijn ouders in de tropen, en zijn fascinatie voor bergen, werkte hij in 1995 in het Lady Willingdon Hospital te Manali in de Indiase Himalaya, waarvan enkele maanden in de nieuw opgezette buitenkliniek in Spiti op de grens met Tibet (opleider Dr. Laji Varghese).

Na in 1996 als AGNIO interne geneeskunde te hebben gewerkt onder de enthousiasmerende supervisie van Dr. Wim van Dorp in het Kennemer Gasthuis te Haarlem, werd van 1997 tot 2002 de opleiding tot kinderarts gevolgd in Máxima Medisch Centrum te Veldhoven (opleider Dr. Walther Tjon a Ten) en het UMC St. Radboud (opleider Prof. dr. R. Sengers). Van 2002 tot 2005 was hij werkzaam op de Kinder Intensive Care van het Radboud (opleider Prof. dr. Hans van der Hoeven), waar begonnen werd met het promotie onderzoek. Sinds 2005 is hij met veel plezier werkzaam als kinderarts-intensivist op de afdeling Pediatrie en Neonatale ICU van Máxima Medisch Centrum te Veldhoven.

De interesse in 3e wereld gezondheidszorg is al die jaren gebleven, en met zijn echtgenote is hij nog frequent betrokken bij het ziekenhuis in Manali, onder andere door er de afgelopen jaren kortere periodes te werken. In de toekomst hoopt de auteur weer voor kortere tijd naar de tropen terug te gaan. Naar de bergen wordt vaker terug gegaan; na een intensieve opleiding heeft de auteur in 2005 het Internationales Alpin Rettungs- und Berg Arzt diploma behaald.

De auteur is getrouwd met Madeline Pikaar met wie hij zijn liefde voor het leven deelt, en zij hebben tot hun geluk 3 kinderen: Ruben, Jelske en Matthijs. Het gezinsleven wordt verrijkt door liefdevolle (groot)ouders en veel vrolijke, dierbare vrienden.