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BIOMARKERS IN ACUTE RESPIRATORY FAILURE

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Academic dissertation

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications. In the text they are referred to by their Roman numerals (I-IV). Articles have been reprinted with the kind permission of their copyright holders.

- I Linko R, Okkonen M, Pettilä V, Perttilä J, Parviainen I, Ruokonen E, Tenhunen J, Ala-Kokko T, Varpula T, the FINNALI Study Group. *Acute respiratory failure in intensive care units. FINNALI: a prospective cohort study.* Intensive Care Med 2009;35:1352–1361
- II Okkonen M, Varpula M, Linko R, Perttilä J, Varpula T, Pettilä V, the FINNALI Study Group. *N-terminal-pro-BNP in critically ill patients with acute respiratory failure: a prospective cohort study.* Acta Anaesthesiol Scand 2011;55:749-757
- III Okkonen M, Lakkisto P, Korhonen AM, Parviainen I, Reinikainen M, Varpula T, Pettilä V, the FINNALI Study Group. *Plasma cell-free DNA in mechanically ventilated patients.* Crit Care 2011;15:R196
- IV Okkonen M*, Gäddnäs F*, Pettilä V, Laurila J, Ohtonen P, Risteli J, Linko R, Ala-Kokko T, the FINNALI Study Group. *Serum markers of collagen synthesis and degradation in acute respiratory failure patients with prolonged hospitalisation.*
* contributed equally
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LIST OF ABBREVIATIONS

AEC	Alveolar epithelial cell
AECC	American-European Consensus Conference
ALI	Acute lung injury
APACHE	Acute physiology and chronic health evaluation
APC	Activated protein C
ARDS	Acute respiratory distress syndrome
ARF	Acute respiratory failure
ATII	Angiotensin II
AUC	Area under receiver operating characteristics curve
BAL	Bronchoalveolar lavage
CC16	Clara cell secretory protein 16
COPD	Chronic obstructive pulmonary disease
DAD	Diffuse alveolar damage
G-CSF	Granulocyte colony-stimulating factor
ICD	International statistical classification of diseases and health problems
ICTP	Type I collagen cross-linked telopeptides
ICU	Intensive care unit
IDI	Integrated discrimination improvement
IL	Interleukin
ITU	Intensive treatment unit
LIS	Lung injury score
MMP	Matrix metalloproteinase
MOD	Multiple organ dysfunction
MV	Mechanical ventilation
NO	Nitric oxide
NRI	Net reclassification improvement
NT-pro-BNP	Aminoterminal pro-brain natriuretic peptide
PAF	Platelet activating factor
PAI-1	Plasminogen activator inhibitor –1
PaO ₂ /FiO ₂ –ratio	The ratio of arterial oxygen pressure and the fraction of inspired oxygen
PAOP	Pulmonary artery occlusion pressure
PAR-1	Protease activated receptor –1
PBW	Predicted body weight
PEEP	Positive end expiratory pressure
PINP	Procollagen type I aminoterminal propeptide
PIIINP	Procollagen type III aminoterminal propeptide

RAGE	Receptor for advanced glycation end products
ROC	Receiver operating characteristics curve
RCT	Randomized controlled trial
SAPS	Simplified acute physiology score
sICAM	Soluble intercellular adhesion molecule
SOFA	Sequential organ failure assessment
SP-A	Surfactant protein –A
SP-D	Surfactant protein –D
sTNFR	Soluble tumor necrosis factor receptor
TF	Tissue factor
TGF- β	Transforming growth factor –beeta
TNF- α	Tumor necrosis factor -alfa
VALI	Ventilator associated injury
vWf	von Willebrand factor

ABSTRACT

Background: Acute respiratory failure (ARF) is the most common type of organ failure leading to the need for intensive care. It is often secondary to acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS). These disorders are characterised by inflammatory reaction and tissue damage. After the inflammatory reaction ceases, it is typically followed by a repair process and restored organ function. In some cases, inflammation continues and leads to an overwhelming repair process with ongoing fibrosis, accompanied by organ dysfunction and eventually a loss of function.

ARF, and especially ALI and ARDS, cause increased morbidity and a need for intensive care. Mortality rates remain high (up to 40%) despite extensive research efforts and increased caution in patient care. Mechanical ventilation, although a lifesaving treatment for ARF, can cause injury to the lungs and amplify the inflammatory process; however the use of a lung protective ventilatory strategy is known to improve the outcome in ALI and ARDS.

Measuring the magnitude of the inflammation, and the repair process, early in the course of disease, would theoretically offer information concerning the outcome. Early identification of patients whose disease process is likely to proceed unfavourably, would help clinicians to optimise their treatment and target novel therapies reasonably. Different biomarkers have been studied in order to understand the process of lung injury, find new therapies, and predict outcome.

The aim of this study was to evaluate the epidemiology of ARF, its treatment, and outcome in Finland, with special interest in biomarkers, and their value in the prediction of mortality.

Patients and methods: Altogether, 958 patients were prospectively included in this study during an eight week period in 2007. The patient cohort comprised adult patients treated in 25 intensive care units with invasive or non-invasive ventilatory support for more than six hours. The whole cohort was studied in order to investigate the epidemiology, treatment methods used, and the outcome of ARF in Finland (Study I). Concentrations of aminoterminal pro-brain natriuretic peptide (NT-pro-BNP) were assessed in 602 patients (Study II), and plasma cell-free DNA in 580 patients (Study III), to evaluate their value as prognostic markers in ARF. Finally, in 68 patients, procollagen type I and III aminoterminal propeptides (PINP, PIIINP) were studied as markers of collagen synthesis and collagen type I cross-linked telopeptides (ICTP) as a marker of collagen I degradation. Their concentrations in longitudinal serum samples were measured in order to evaluate their evolution in

ARF patients with and without multiple organ dysfunction (Study IV).

Main results: Of all 2473 patients admitted to intensive care units, 958 (39%) were treated with ventilatory support for more than six hours. The estimated incidence of ARF treated in the ICUs over this eight-week period was 149.5 / 100 000 per year. Median tidal volume per predicted body weight was higher than recommended, median 8.7 ml/kg. Overall mortality at 90 days was 31%.

Both plasma NT-pro-BNP and cell-free DNA concentrations were highly increased in ARF patients, and levels were higher in 90-day non-survivors than survivors. NT-pro-BNP values were higher in patients with cardiac condition, and with renal dysfunction. Plasma NT-pro-BNP was a moderate predictor of 90-day mortality. Plasma cell-free DNA levels were higher in non-operative patients with infection than without infection. Baseline values of plasma DNA were independent predictors of 90-day mortality, but the discriminative power was poor.

NT-pro-BNP and plasma cell-free DNA remained independent predictors for 90-day mortality in logistic regression analysis including both biomarkers. The mortality was highest in those patients, in whom both biomarkers were over their separate cut-off values.

PIIINP levels increased over time in ARF patients with prolonged hospitalization. PINP levels also increased, but the degradation exceeded the synthesis during first week of ARF. The markers of collagen metabolism showed recovery back to baseline at day 21. None of the markers of collagen metabolism did associate with multiple organ dysfunction.

Conclusions: The estimated incidence of ARF treated in the ICU was 149.5 / 100 000 per year in Finland. Ventilatory support for more than six hours was used in 39% of all ICU patients. The 90-day mortality in ARF was 31%. Lung protective ventilatory strategy was adapted moderately. Plasma NT-pro-BNP and cell-free DNA were commonly high in ARF patients, and both were independent predictors of 90-day mortality. However, plasma NT-pro-BNP concentrations were affected by renal failure, and the sensitivity and specificity of plasma cell-free DNA values were poor. Combined use of these biomarkers, however, may increase their clinical value in mortality prediction. The degradation of collagen type I and synthesis of collagen type III increased during the first week in surviving patients. Collagen metabolism approached normal levels at three weeks.

Keywords: Acute respiratory failure, intensive care, mortality, outcome, N-terminal-pro-brain natriuretic peptide, plasma cell-free DNA, collagen, procollagen propeptide

1 INTRODUCTION

Acute respiratory failure (ARF) is the most common organ failure reported in intensive care unit patients (Flaatten et al. 2003). In fact, according to most reports, 45-60% of all patients admitted to intensive care units require respiratory support (Demoule et al. 2006, Roupie et al. 1999, Vincent et al. 2002). Since almost all diseases and organ dysfunctions can lead to insufficient pulmonary ventilation, pulmonary oxygenation, or both, triggers for ARF are very heterogeneous. They can also initiate an inflammatory reaction that leads to acute lung injury (ALI) or its more severe form, acute respiratory distress syndrome (ARDS). ALI and ARDS are important precursors of ARF and share common pathophysiological features. They are characterised by inflammatory reaction and tissue damage, histologically depicted as diffuse alveolar damage (DAD), resulting in a clinical picture of deteriorating oxygenation and/or ventilation. The inflammatory reaction often ceases, and is followed by repair process and restored organ function. Nonetheless, inflammation does continue in some cases, where it leads to an overwhelmed repair process with ongoing fibrosis accompanied by organ dysfunction and, in the worst-case scenario, loss of function.

Introducing ventilatory support in ARF can be life saving, assuming that the disorder leading to respiratory failure is temporary and can be treated. Ventilatory support, however, is known to be potentially injurious. Since the first landmark studies (Dreyfuss et al. 1985, Webb et al. 1974), numerous other experimental studies have shown that lung injury can ensue in previously healthy lungs after implementing injurious ventilatory settings. More recent clinical studies have also supported these findings (Gajic et al. 2005, Pinheiro de Oliveira et al. 2010). A lung protective ventilatory strategy for ALI and ARDS patients, aiming to avoid overdistension and cyclic opening and closing of alveoli, has been established a decade ago, based on a large multicentre study (ARDSNet 2000). Mortality has been suggested to have decreased to some extent, but still remains high at around 40% (Esteban et al. 2008, Zambon et al. 2008).

A detailed understanding of ARF is lacking, given that typically non-homogeneous studies of very different patient materials are reported, due to lack of standard definition. Instead, the most severe forms of ARF, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), have been the research target of interest over the last decades. ARDS as a clinical syndrome was first described in 1967 by Ashbaugh and colleagues (Ashbaugh et al. 1967). Standard definitions for ALI and ARDS were formulated by American-European Consensus Conference (AECC) in 1994 (Bernard et al. 1994). Due to the lack of a standard definition, the

need for mechanical ventilation has been commonly used as a definition of ARF in epidemiological studies.

Preventing ALI is a desirable goal, since it causes increased morbidity and mortality, and its treatment is expensive. Early identification of those patients who are at a high risk of developing severe lung injury would help clinicians to optimise their treatment and to target novel therapies. In most cases, the development of severe lung injury is multifactorial, including genetic predisposition. Thus, in order to prevent ALI, it is essential to focus on a large and unselected patient population with existing risk factors for ALI. Patients treated with ventilatory support represent a plausible study population, since after the original injurious incident, ventilatory treatment can be considered a second hit, increasing the risk of ALI. Due to the inflammatory nature of ALI, encompassing numerous elements of inflammatory and coagulation cascades, a myriad of possible biomarkers for ALI exist. Biomarker studies have not only increased the knowledge of pathophysiological pathways, but also yielded some biomarkers that have shown promise in the diagnostics and outcome prediction for ALI and ARDS.

The epidemiology of ARF and its treatment in Finland have not been studied previously, thus a clear opening for a new investigation is evident. Furthermore, measuring the magnitude of the inflammation and the repair process would be advantageous in theory. Early identification of those patients, whose disease process is likely to proceed unfavourably, would help clinicians to optimise their treatment and target novel therapies reasonably. In addition, it would be of great interest to find new and individual tools for prognostic purposes. The aim of this thesis was to study the epidemiology of ARF, its treatment, and outcome in Finland, with special interest in biomarkers, and their value in the prediction of mortality.

2 REVIEW OF LITERATURE

2.1. THE EPIDEMIOLOGY OF ARF, ALI AND ARDS

2.1.1. DEFINITIONS

2.1.1.1. ARF

Respiratory failure may be described as an incapability of the respiratory system to maintain sufficient function with regard to demands. The respiratory system has two functions, oxygenation of blood and elimination of carbon dioxide. The failure of either or both of these functions results in hypoxemia and/or respiratory acidosis (Roussos et al. 2003). If the onset of the failure is acute, the compensatory mechanisms do not have time to work and a life threatening situation ensues.

Numerous illnesses and pathological states can result in acute respiratory failure. Primary pulmonary diseases may directly affect gas exchange, the control of breathing may be disabled by central nervous system disease, and systemic inflammatory reaction may cause increased oxygen consumption or increased production of carbon dioxide resulting in increased work of breathing and respiratory muscle fatigue. This miscellaneous background and the symptom-like nature of ARF most probably is one reason why a standard definition is lacking.

Most studies have only included patients with ALI or ARDS, but these syndromes cover a minority of ARF patients. In a Nordic multicentre study, where ARF was defined as invasive mechanical ventilation for more than 24 hours, ALI or ARDS definitions were fulfilled in 287 (23%) of 1231 ARF patients (Luhr et al. 1999), in agreement with proportions reported by others (Villar et al. 2007). Even smaller proportions of ALI patients have been found, when mechanically ventilated patients have been evaluated (Esteban et al. 2008, Roupie et al. 1999). Widely differing definitions of ARF have been used, some including criteria for oxygenation (Flaatten et al. 2003, Vincent et al. 2002), and some demanding invasive ventilation (Lewandowski et al. 1995, Luhr et al. 1999).

The use of variable inclusion criteria has resulted in wide range of study results. Table 1 presents epidemiological studies with different patient populations included, and also the proportions of ALI/ARDS from study patients.

Table 1. Epidemiological studies of ARF or ALI/ARDS

Study	Setting	ICU admissions	Study patients * Inclusion criteria entitled as ARF
Lewandovski 1995	72 ICUs in Berlin 1991, 8 weeks Prospective	NA	* Intubation + MV \geq 24 h (\geq 14 years)
Roupie 1999	36 French ICUs 1996, 14 days Prospective	976 (424 with MV)	PaO ₂ /FiO ₂ < 300 mmHg + MV + acute unset (NIV included)
Luhr 1999	132 ICUs in 3 Nordic countries 1997, 8 weeks Prospective	13 346	* Intubation + MV \geq 24 h
Behrendt 2000	Register data analysis, 1994, 1 year Retrospective	NA	* ICD-9-CM code for acute respiratory distress or failure + MV (>5 years)
Vincent 2002	40 ICUs in 16 countries, 1995, 1 month Prospective	1449	* PaO ₂ /FiO ₂ < 200 mmHg + MV (\geq 12 years) (NIV included)
Bersten 2002	21 adult ICUs in 3 Australian states 1999, 8 weeks Prospective	1977	ALI/ARDS (AECC criteria)
Esteban 2002	361 ICUs in 20 countries, 1998, 28 days Prospective	15 757	MV > 12 h (NIV included)
Flaatten 2003	One ICU in Norway, 2000-2002, 2.5 years Prospective	832	* SOFA 3 or 4 at least on 2 days (>16 years) (NIV included)
Goss 2003	20 hospitals in United States 1996-1999 Prospective	NA	ALI/ARDS (AECC criteria)
Brun-Buisson 2003	78 ICUs in 10 European countries 1999, 2 months Prospective	6522	ICU stay > 24 h or ALI/ARDS at admission
Rubinfeld 2005	21 hospitals in one county in USA 1999-2000, 12 months, Prospective	NA (6235 admissions with MV)	MV > 24 h (> 6 months of age)
Esteban 2008	349 ICUs in 23 countries 2004, 1 month Prospective	19 505	MV > 12 h (NIV included)
The Irish Critical Care Trials Groups 2008	14 ICUs in Ireland, 10 weeks Prospective	1029 (728 with MV)	ALI/ARDS (AECC criteria)

AECC, American-European Consensus Conference; ALI, Acute lung injury; ARDS, Acute respiratory distress syndrome; ARF, Acute respiratory failure; ICD-9-CM, The International Classification of Diseases, Ninth Revision, Clinical Modification; ICU, Intensive care unit; LIS, Lung injury score; MV, Mechanical ventilation

Patients	ALI/ARDS of study patients	Incidence /100 000	Hospital mortality	Comment
508	17 (3.6%) Severe lung injury (LIS > 2.5)	ARF 88.6 Severe lung injury 3.0	42.7% (ICU) 59% ARDS (LIS>2.5)	Severe lung injury defined as LIS > 2.5
213	67 ARDS (31.5% of study patients and 15.8% of MV patients)	NA	40% (28 d) 60 % ARDS	49% of patients admitted from wards to ICU
1231	287 (23%)	ARF 77.6 ALI 17.9 ARDS 13.5	ARF 41% * ALI (not ARDS) 42.2% ARDS 41.2%	* 90-day mortality
61 223	NA	ARF 137.1	35.9%	Based on hospital discharge data
458 at adm. + 352 at ICU	NA	NA	34%	23% postoperative patients
(NA)	168 (8.5%) ALI/ARDS 148 (7.5%) ARDS	ALI 34 ARDS 28	32% overall (28 d) 15% ALI, not ARDS 34% ARDS	Barotrauma in pulmonary ALI 16%, in non-pulm. 1.5%, p<0.001
5183	231 (4.5%)	NA	39.2%	PaO ₂ /FiO ₂ independent predictor of death
392 at adm. + 137 at ICU	NA	NA	32.9% 14.7% single organ ARF	Gradual increase in mortality with number of failing organs
(NA)	7455 ALI/ARDS	64	NA	Based on screened patients in ARDSNet study (ARDSNet 2000)
2768	463 (16.1%)	NA	49.4% ALI, not ARDS 57.9% ARDS	Large variation in ARDS prevalence in different centres
4251	1113 (26%) ALI 828 (19%) ARDS	ALI 78.9 ARDS 58.7	38.5% ALI, including ARDS 41.1% ARDS	Only 34% discharged to home
4968	198 (4%) ARDS	NA	37% all patients 63% ARDS	A follow-up study of a previous epidemiological study (Esteban et al. 2002)
196	196 ALI/ARDS (19% of admissions and 27% of MV patients)	NA	32% (ICU)	PaO ₂ /FiO ₂ independent predictor of death

2.1.1.2. ALI and ARDS

Whilst the first descriptions of lung oedema appearing without heart failure were written in the early 1800's, ARDS was first described by Ashbaugh and colleagues in 1967 (Ashbaugh et al. 1967). They described twelve patients with clinical symptoms of severe dyspnoea and cyanosis associated with diffuse alveolar infiltrates seen in the chest x-ray; their symptoms were resistant to usual respiratory therapy approaches. Two decades later, an attempt to define lung injury was made by Murray and colleagues (Murray et al. 1988). Their definition included three parts: time frame (acute or chronic), severity of the lung injury, and the cause of the lung injury. The severity of the lung injury was assessed by lung injury score (LIS), which included the level of positive end expiratory pressure (PEEP) and respiratory system compliance, in addition to the severity of the oxygenation problem, and the number of quadrants affected in the chest x-ray. This definition has been used in some interventional studies (Peek et al. 2009), but has not been adopted widely in epidemiological studies as a diagnostic criteria. The lung injury score, however, has been favoured as a quantitative index of ALI severity.

In 1994, the AECC formulated diagnostic criteria for ALI and ARDS (Bernard et al. 1994), presented in Table 2. The purpose was to standardise the patient populations included in clinical trials, since the definitions, thus patients and results, in earlier studies had varied widely.

Table 2. The diagnostic criteria of ALI and ARDS according to AECC (Bernard et al. 1994).

Acute onset
Bilateral infiltrates in chest x-ray
Absence of left atrial hypertension (PCWP < 18 cmH ₂ O or clinical exclusion)
PaO ₂ /FiO ₂ ≤ 300 mmHg (≤ 40 kPa) for ALI and ≤ 200 mmHg (≤ 27 kPa) for ARDS

AECC, American-European Consensus Conference; ALI, Acute lung injury; ARDS, Acute respiratory distress syndrome; kPa, kilopascal; PCWP, pulmonary capillary wedge pressure

These criteria have been criticised for many reasons. First, ventilatory settings are not taken into account, although they are known to have a significant effect on oxygenation and, thus, the fulfilment of the criteria. In a study by Villar and colleagues, 170 patients met ARDS criteria at baseline (Villar et al. 2007). After adjustments in the PEEP level and in the inspired oxygen fraction, only 99 (52%) fulfilled ARDS criteria, while 55 patients had shifted to the group fulfilling ALI criteria and 16 patients did not meet the ALI/ARDS criteria at all. Second, the ratio of arterial oxygen pressure and the fraction of inspired oxygen (PaO₂/FiO₂ -ratio),

which is used as a measure of oxygenation in AECC–criteria, can be manipulated by simply changing the inspired oxygen fraction (Aboab et al. 2006, Karbing et al. 2007).

Additionally, AECC criteria do not necessarily correlate with patients' clinical status. In a recent study, 715 patients treated in respiratory wards were evaluated for ALI/ARDS criteria (Quartin et al. 2009). Of these 715, 474 (66%) had acute infiltrates in their chest x-ray, 62 (9%) fulfilled the criteria for ALI, and 15 (2%) for ARDS. Patients with ALI criteria displayed a 90-day mortality of only 12%, in contrast to a 10% mortality rate for patients not fulfilling the criteria. This result suggests that the criteria currently used to evaluate for ALI/ARDS are neither sensitive nor specific enough to reliably find patients with acute lung injury. Indeed, in an autopsy study of 133 patients who had been diagnosed to have ARDS, the characteristic histological picture of DAD, a gold standard in the diagnosis of ARDS, was seen only in 119 patients (Esteban et al. 2004). The remaining 14 patients with a clinical diagnosis of ARDS were diagnosed to have suffered from pneumonia, alveolar haemorrhage, pulmonary oedema or embolism, and fibrosis due to chemotherapy. Sensitivity and specificity were calculated for AECC criteria, and were found to be only moderate, with a sensitivity of 75% (95% confidence interval [CI], 66% to 82%) and specificity 84% (95% CI, 79% to 88%) in all patients. In patients demonstrating clinical risk factors, the values remained somewhat lower, both were 75%. In contrast, for patients with non-pulmonary risk factors, both sensitivity and specificity were higher, signifying the importance of underlying pathophysiology.

Despite the obvious weaknesses in the criteria used to define ALI, and continuing suggestions to refine them (Lewandowski 1999, Rumbak et al. 2009), inclusion criteria in practically all studies concerning ALI are based on those defined in table 2.

2.1.2. INCIDENCE

Due to inconsistencies in its definition, studies concerning ARF and its incidence are inconsistent. Given that ALI/ARDS patients are defined as a proportion of ARF/MV patients in many studies, the inclusion criteria used for ARF has great impact in this estimate (Table 1). The ARF incidence rates reported have ranged from 77.6 / 100 000 per year in a Nordic study covering Sweden, Denmark, and Iceland (Luhr et al. 1999) to 137.1 / 100 000 per year in a retrospective register data analysis (Behrendt 2000). Correspondingly, the incidence numbers for ARDS (AECC criteria) have varied from 4.9 / 100 000 per year in a 3-year retrospective analysis in one hospital (Valta et al. 1999) to 58.7 and 64 / 100 000 per year in more recent studies from the United States (Goss et al. 2003, Rubenfeld et al. 2005).

Lower incidences of 1.5 to 3.5 / 100 000 per year have been reported with a more strict ARDS definition (Villar et al. 1989) and 3.6 / 100 000 per year with 'severe lung injury' defined by LIS score (Lewandowski et al. 1995).

2.1.3. OUTCOME

Large variation is seen in both the clinical outcome of ARF, ALI, and ARDS, and their incidence. A crude mortality rate of 35-40% is reported for ARF in most studies (Behrendt 2000, Luhr et al. 1999, Roupie et al. 1999), and 30-60% for ALI/ARDS (Bersten et al. 2002, Brun-Buisson et al. 2004, Rubenfeld et al. 2005).

Whether the mortality of ALI/ARDS has been decreasing over decades is under debate, since the results have not been consistent and the interpretations have also differed, as reported in two recent systematic reviews (Phua et al. 2009, Zambon et al. 2008). Zambon and Vincent analysed ALI/ARDS studies from 1994 (when the AECC criteria were published) to 2006, and included 72 studies with more than 30 patients (Zambon et al. 2008). They found that mortality rates showed wide variation from 15% (28-day mortality) to 72% (hospital mortality) between studies, the overall mortality being 43%. Mortalities reported in studies varied from ICU to 60-day mortality. A constant reduction in the overall and hospital mortalities, but not in others, was noticed over time. In another systematic review, 89 studies including at least 50 patients with ALI/ARDS, were evaluated (Phua et al. 2009). Studies were pooled according to a publishing year, thus were published before or after the AECC consensus criteria in 1994 (Bernard et al. 1994), and also according to study design, namely observational versus randomized controlled trials (RCT). The overall mortality in all studies was 44.3%. Mortality was noticed to decrease over time in observational studies conducted before year 1994, however from 1994 to 2006 no changes in mortality over time were perceived. In the era from 1994 to 2006, the mortality rate differed significantly according to study design, being 44% in observational, and 36% in randomized controlled trials (RCT).

2.1.3.1 *Parameters affecting outcome*

Diverse definitions used in earlier studies are partly responsible for variable results, but differences in the local treatment protocols, organizational factors, admission policies, and also populations may also have had an effect on differing results. When the effect of closed versus open ICUs on hospital mortality was evaluated, the mortality was significantly lower in ALI patients in the closed ICUs (Treggiari et al. 2007). In open ICUs, 31% of the patients were ventilated with tidal volumes

known to be injurious (over 12 ml/kg), compared to 10% in closed ICU, which may be a plausible explanation for the increased mortality in open ICUs. Another study has shown that higher hospital volume, thus the higher number of patients treated, is associated with decreased mortality in mechanically ventilated patients (Kahn et al. 2006).

Predisposing factors for ALI and ARDS also have an impact on the outcome. The mortality in ALI/ARDS has been reported to be highest in sepsis induced ALI (Brun-Buisson et al. 2004, Doyle et al. 1995). Trauma patients, in contrast, have a better prognosis, which is not entirely explained by the baseline characteristics, but has been shown to associate with lower levels of biomarkers for both epithelial and endothelial injury (Calfee et al. 2007). Two retrospective analyses have suggested that race may impact the outcome (Erickson et al. 2009, Moss et al. 2002), however another study found no racial difference in trauma patients regarding ALI development, its severity, or mortality (Brown et al. 2011). Nonetheless, other genetic factors may still have an effect on survival (Marshall et al. 2002)

The impact of the severity of the oxygenation problem on mortality has varied in earlier studies. In a Nordic multicentre study, mortality rates were comparable in different groups, where 90-day mortality was 41% for ARF (including ALI and ARDS patients), 42% for ALI (not including ARDS), and 41% for ARDS patients (Luhr et al. 1999). In another study, the long-term outcome in critically ill trauma and sepsis patients did not differ, whether the ARDS was diagnosed or not (Davidson et al. 1999). Some other studies, on the other hand, have shown significant differences in outcome according to the severity of lung injury (Esteban et al. 2002, IrishCriticalCareTrialsGroup 2008, Roupie et al. 1999, Villar et al. 2007). Overall, PaO₂/FiO₂ – ratio on the first day of ALI/ARDS has not been shown to be an independent predictor of mortality (Ware 2005).

Acute respiratory failure is an infrequent cause of death in ARDS patients. A landmark study by Montgomery and colleagues, reported that in only 16% of patients with ARDS, death was caused by acute respiratory failure (Montgomery et al. 1985). In most cases, when occurring after the first days of disease onset, death is related to other organ failures, especially to multiple organ failure (Ferring et al. 1997). Mortality has been shown to associate more with other organ dysfunctions than respiratory failure per se (Flaatten et al. 2003). Flaatten and colleagues reported hospital mortality as low as 15% in acute respiratory failure, as a single organ failure, however this increased as organ failures compounded. A comparable mortality rate of 17% with a single organ ARF has been reported, however patient number with ARF as a single organ failure was only 24 (Pettila et al. 2002). In addition, the combination of ARF with any other organ failure led to a steep increase in mortality, ranging from 51% with circulatory failure, to 75% with hematologic failure. Other organ dysfunctions have also independently predicted death in studies on ALI/ARDS (Brun-Buisson et al. 2004, Doyle et al. 1995).

2.2. MECHANISMS OF ACUTE LUNG INJURY AND ASSOCIATED BIOMARKERS

Lung injury may commence with diverse early pathophysiology, caused by either direct or indirect mechanisms. These mechanisms each constitute approximately half of the cases of ALI/ARDS (Bersten et al. 2002, Luhr et al. 1999, Villar et al. 2007). The site of injury is alveolar epithelium in direct pulmonary injury as in aspiration and pneumonia, and vascular endothelium in indirect extrapulmonary injury, as in sepsis, trauma, massive transfusion. Both situations, or often a combination of them, result in increased vascular permeability, interstitial fluid accumulation, and inflammatory cell migration in alveolar spaces (Ware et al. 2000). Air spaces flood with protein rich oedema fluid, and activated neutrophils and macrophages release inflammatory mediators and proteases, which further amplify the inflammatory process and injury. Histologically acute lung injury is characterised by DAD (Castro 2006, Katzenstein et al. 1976). This includes type II alveolar epithelial cell (AEC) loss, leading to surfactant deficiency and functional impairment, while destruction of type I AECs results in impairment of gas exchange.

The inflammatory and regenerative processes take place in the denuded alveolar epithelium (Pugin et al. 1999). Epithelium disruption triggers the activation of coagulation cascades, leading to a subsequent activation of tissue fibroblasts. Myofibroblasts are stimulated, for example by activated transforming growth factor (TGF)- β , to secrete procollagens (Scotton et al. 2007) (Figure 1). Excess collagen, in turn, is degraded by matrix metalloproteinases (MMPs), as are other extracellular matrix structural components, thus MMPs are important in the regulation of tissue remodelling. They also participate in the regulation of inflammation and immunity, participating in both pro- and anti-inflammatory actions. In fact, proinflammatory mediators tumor necrosis factor (TNF)- α and TGF- β have been reported to induce the action of MMP-2 and MMP-9 (Han et al. 2001a, Han et al. 2001b).

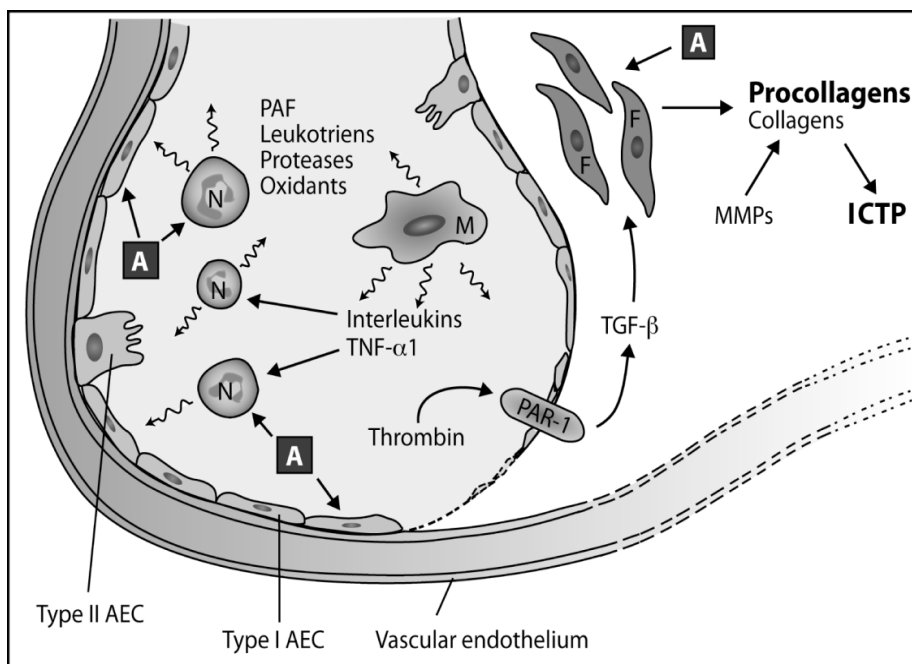


Figure 1. Simplified mechanisms of acute lung injury, drawn by Helena Schmidt according to draft by Marjatta Okkonen.

A, apoptosis; AEC, alveolar epithelial cell; F, fibroblast; ICTP, collagen type I cross-linked telopeptides; M, macrophage; MMPs, matrix metalloproteinases; N, neutrophil; PAF, platelet activating factor; PAR-1, protease activated receptor-1; TNF- α , tumor necrosis factor- α ; TGF- β , transforming growth factor- β

Although many features of ALI are common to both direct pulmonary and indirect extrapulmonary insults, some differences exist. Morphological changes in lung parenchyma, changes in lung mechanics, and expression of MMP-9, have been shown to be different in pulmonary and extrapulmonary lung injury in a mouse model (Santos et al. 2006). Changes in lung parenchyma morphology (collagen deposits, fibroblast appearance) disappeared more rapidly in non-pulmonary injury. Additionally, in pulmonary, but not extrapulmonary injury, elastic fibres increased in addition to collagen fibres. A human study evaluating 21 ARDS patients has shown that the lung mechanics differ significantly in pulmonary and extrapulmonary injury (Gattinoni et al. 1998), in accordance with experimental investigations. Lung elastance was significantly higher in pulmonary than in extrapulmonary ARDS, and the chest wall elastance in turn, was higher in extrapulmonary ARDS. The effect of increasing PEEP level was also different, lung elastance increased and no recruitment was observed in pulmonary ARDS. The opposite effect was noticed in extrapulmonary ARDS, with a decrease in lung elastance and significant lung recruitment. These results clearly suggest that transpulmonary pressure is higher

in ARDS caused by pulmonary insult, and thus the risk of further injury caused by ventilatory treatment may be significant.

Due to the inflammatory nature of ALI, a range of elements in biological cascades may have potential as biomarkers. Numerous markers have been studied in experimental studies, but considerably fewer in the clinical setting, and still fewer in multicentre studies. Large multicentre studies evaluating biomarkers are widely based on the patient population recruited to one randomised, controlled study evaluating the effect of low versus traditional tidal volumes on outcome in ALI/ARDS patients (ARDSNet 2000). Table 3 presents these studies with particular focus on the statistical methods reported in the publications. Most of these studies report the association of the biomarker with the outcome measure. Most studies also report the results of the logistic regression analysis, performed to exclude the effect of the other risk factors, and to measure if the biomarker has an independent effect on outcome. Results concerning the clinical utility of a biomarker are, however, not reported. A test widely used and recommended for this purpose is the area under the receiver-operating-characteristic (ROC) curve (AUC) analysis. This method has been used widely in order to measure the discriminative value and, thus, the clinical utility of a biomarker (Ray et al. 2010). More advanced statistical solutions, namely the net reclassification improvement NRI and the integrated discrimination improvement IDI, have been proposed in order to further improve the assessment of new biomarkers (Cook et al. 2009, Pencina et al. 2008). These indices are based on stratification into clinical risk categories, and have already been reported in a biomarker study (Wang et al. 2011). Although some weaknesses in reports from multicentre biomarker studies exist, the strength of these studies is a large patient population, and especially a controlled study design concerning ventilatory treatment. No systematic review of the biomarkers in ALI/ARDS have been performed, but recently Levitt and colleagues have published an analytical review (Levitt et al. 2009).

Table 3. Multicentre studies evaluating biomarkers of ALI.

Study	Marker(s)	N	Non-survivors	Statistics (yes/no)				Result	
				Mean or median	Mann-Whitney, Wilcoxon or Kruskal-Wallis	AUC	logistic regression		IDI
Eisner 2003	SP-A SP-D	565	195	Median	+	-	+	-	SP-A: not predictive of outcome SP-D: higher plasma levels associated with mortality, low Vt strategy attenuated levels
Ware 2004	von Willebrand factor (vWf)	559	193	Mean	+	-	+	-	No difference in vWf levels in patients with 1) sepsis or not 2) low or traditional Vt
Parsons 2004	sTNF α I and II	562	196	Median	+	-	+	-	sTNF α I and II independent predictors of mortality Reduction in sTNF α I levels by low Vt ventilatory strategy
Parsons 2005	IL-6 IL-8 IL-10	781	276	Median	+	-	+	-	Highest levels in sepsis and pneumonia, lowest in trauma Lower Vt group: greater decrease in IL-6 and IL-8
McClintock 2006	U-desmosine	579	NA	Mean	+	-	+	-	Higher urine desmosine to creatinine ratio independent predictor of death
Ware 2007	protein C PAI-1	779	NA	Median	+	-	+	-	Protein C and PAI-1 both independently predict mortality Synergistic interaction for mortality risk
McClintock 2007	U-NO	566	NA	Median	+	-	+	-	Higher levels of U-NO independently predicted lower mortality Greater increase in values (D0-D3) in low Vt strategy group
Calfee 2008	RAGE	767	NA	Median	+	-	+	-	High levels associated with mortality only in high Vt group
Calfee 2009	sICAM-1	778	NA (35%)	Median	+	-	+	-	High and increasing sICAM-1 associate with poor outcome

IL, interleukin; NA, not available; PAI-1, plasminogen activator inhibitor-1; RAGE, receptor for advanced glycation end products; sICAM-1, soluble intercellular adhesion molecule-1; SP-A/D, surfactant protein A/D, sTNF α , soluble tumor necrosis factor receptor; U-NO, urine nitric oxide; Vt, tidal volume

2.2.1. PULMONARY OEDEMA

Pulmonary oedema is a distinguishing feature of an acute phase of ALI (Sibbald et al. 1985, Ware et al. 2000). Instead of cardiac insufficiency and increased intravascular pressure, increased alveolar permeability is the major component in the pathogenesis (Staub 1978). Impaired alveolar fluid clearance has also recently been noticed (Ware et al. 2001b). The extent of oedema formation, as measured by extravascular lung water, has been shown to correlate with mortality in ARDS (Sakka et al. 2002). The reduction of pulmonary capillary wedge pressure, as a result of diuretic treatment, has also shown to correlate with improved outcome in a retrospective study (Humphrey et al. 1990). More recently, a randomized, controlled study evaluating conservative versus liberal fluid management strategy in ALI patients has been published (Wiedemann et al. 2006). Therein, conservative fluid management reduced the ventilator time and ICU length of stay without increasing other organ failures, such as acute kidney injury, in patients with established ALI/ARDS after shock had reversed. This result emphasizes the importance of fluid overload in lung injury, and suggests the possible role of brain natriuretic peptide (BNP) as a biomarker.

2.2.1.1. *N-terminal pro-brain natriuretic peptide (NT-pro-BNP)*

BNP belongs to a family of neurohormones and has several circulatory and homeostatic activities. It is vasodilatory and diuretic, antagonises the renin-angiotensin-aldosterone system, and inhibits the sympathetic nerve system. Myocardial cells synthesize and excrete BNP mainly in response to myocardial wall stretch and volume overload. Endothelin-I and angiotensin (AT)II are the most powerful inducers of BNP release (de Bold et al. 1996). Inflammatory pathways and endothelial dysfunction have also been suggested to be interacted with BNP (Clerico et al. 2006). BNP is a prohormone, which is proteolytically cleaved to yield active C-terminal and inactive N-terminal parts in a one to one ratio during its release from cells. NT-pro-BNP is released in equimolar amounts to active BNP, and thus, can be used to measure its levels. The advantage of NT-pro-BNP over BNP is its stability during sampling and storage (Clerico et al. 2007, Pemberton et al. 2000). NT-pro-BNP is excreted mainly by kidneys, thus renal failure is a potential confounding factor in its use as a biomarker in lung failure (Forfia et al. 2005a). The levels of NT-pro-BNP are significantly higher than the levels of BNP but have shown to correlate with each other (Masson et al. 2006).

Numerous cardiac and non-cardiac conditions cause an increase in NT-pro-BNP levels (Zakynthinos et al. 2008). BNP has shown powerful prognostic value on outcome in both acute and chronic heart failure (Di Somma et al. 2010, Jarai et al. 2009, Latini et al. 2004) and in acute coronary syndrome (James et al. 2003, Jarai et al. 2005).

High elevations in natriuretic peptide levels have been reported also in non-cardiac patients. Most widely studied after cardiac patients, are patients with sepsis (Brueckmann et al. 2005, Charpentier et al. 2004). Some studies have reported a lack of ability of NT-pro-BNP to differentiate between cardiac and non-cardiac pulmonary oedema patients (Bajwa et al. 2008, Jelic et al. 2005), but contradictory results have also been published (Karpaliotis et al. 2007). Recent studies have shown some prognostic value for NT-pro-BNP in hypoxic ARF patients (Karpaliotis et al. 2007), and in patients with severe sepsis or septic shock (Karpaliotis et al. 2007, Varpula et al. 2007). In more unselected populations of critically ill patients, NT-proBNP has been shown to be an independent predictor of mortality (Almog et al. 2006, Meyer et al. 2007). The value of NT-pro-BNP, however, has remained unclear in the critical care setting (Christenson 2008). Recently, association between elevated levels of NT-pro-BNP and unfavorable outcome has been suggested in mechanically ventilated (Kotanidou et al. 2009) and ARDS (Bajwa et al. 2008) patients. Studies evaluating BNP or NT-pro-BNP in mechanically ventilated, ARF, or ARDS patients are presented in Table 4.

Table 4. Studies evaluating brain natriuretic peptides (BNP or NT-pro-BNP) in mechanically ventilated, ARF, or ARDS patients.

	Patients population	Number of patients / non-survivors	Study aim	Result
Jelic 2005	Hypoxic respiratory failure	41/17 (41%) (30-day)	1) can NT-pro-BNP differentiate between patients with high vs. low PAOP pulmonary oedema 2) does NT-pro-BNP predict 30-day mortality	1) NT-pro-BNP does not correlate with PAOP 2) no value in mortality prediction
Karpaliotis 2007	Hypoxic respiratory failure (PaO ₂ /FiO ₂ <300 mmHg)	80/ NA	1) does BNP assist in diagnostics of cardiogenic vs. non- cardiogenic pulmonary oedema? 2) utility of BNP in prognostic evaluation	1) BNP is useful in excluding cardiogenic pulmonary oedema 2) associates with hospital mortality in all patients
Berdal 2008	MV > 48 hours	70/25 (36%) (30-day)	Prognostic value of NT-pro-BNP on 30-day mortality	Independent predictor of mortality, AUC 0.74
Bajwa 2008	ARDS	177/70 (40%) (60-day)	1) to evaluate NT-pro-BNP levels in ARDS patients 2) to examine the diagnostic and prognostic value	1) elevated levels found in ARDS, median 3180 pg/ml 2) no diagnostic value, independent predictor of 60-mortality
Kotanidou 2009	Unselected, non-cardiac ICU patients, all with MV	233/98 (42%) (ICU)	to evaluate prognostic value of NT-pro-BNP	independent predictor of ICU mortality

ARDS, acute respiratory distress syndrome; ARF, acute respiratory failure; ICU, intensive care unit; MV, mechanical ventilation; NA, not available; NT-pro-BNP, aminoterminal pro-brain natriuretic peptide; PAOP, pulmonary artery occlusion pressure

2.2.2. MECHANICAL INJURY

Ventilatory support is a life saving treatment in ARF, but it can also augment the existing lung injury and even cause injury to previously healthy lungs. This injury, referred as 'ventilator associated lung injury' (VALI), may originate simply due to mechanistic reasons, since lungs exhibit a non-homogeneous structure with aeration differing widely under mechanical ventilation. This can cause powerful traction forces to occur in the intermediate region between areas of different aeration, as shown by Mead and colleagues in their classical study (Mead et al. 1970). Early animal studies have shown that ventilation with high tidal volumes and high inspiratory pressures is injurious to lungs (Dreyfuss et al. 1985, Kolobow et al. 1987, Tsuno et al. 1991, Webb et al. 1974). In humans, studies have mostly focused on patients with the diagnosis of ALI or ARDS. The first observations of reduced mortality, when tidal volumes and inspiratory pressures had been limited in ARDS patients, were published by Hickling and colleagues (Hickling et al. 1990, Hickling et al. 1994). These studies were retrospective and observational and had no control groups, but raised much interest in the issue.

The issue of injurious ventilation was further studied by including 44 patients in a RCT evaluating the impact of ventilatory settings on inflammatory mediators (Ranieri et al. 1999). Results confirmed that ventilation with high tidal volume (11 ml/kg) and inspiratory pressure (31 cmH₂O) caused an increase in both bronchoalveolar lavage (BAL) fluid and plasma levels of TNF- α , interleukin(IL)-6, and TNF receptors. Lung protective ventilation, on the contrary, resulted in a decrease of these mediators. These results support the hypothesis that ventilator treatment may induce and augment the systemic inflammatory response and cause remote organ failure that may ultimately lead to multiple organ failure. Concurrently, four randomised, controlled studies evaluating the effect of tidal volume limitation on outcome in ARDS patients were published (Amato et al. 1998, Brochard et al. 1998, Brower et al. 1999, Stewart et al. 1998). One study showed a large decrease in the 28-day mortality in the low tidal volume group (38%) when compared to traditional tidal volume group (71%) (Amato et al. 1998), but high mortality in the control group has raised criticism. Three other studies, with enrolled patient numbers varying from 52 to 120, however, failed to show any outcome benefit associated with low tidal volume strategy (Brochard et al. 1998, Brower et al. 1999, Stewart et al. 1998). More recently, a large randomized, controlled study comparing low (6 ml/kg predicted body weight, PBW) and traditional (12 ml/kg PBW) tidal volumes in ARDS was performed, with the results showing a significant decrease in hospital mortality in the low tidal volume group (31%) versus the traditional tidal volume group (40%) (ARDSNet 2000). These somewhat contradictory results were discussed in a meta-analysis (Eichacker et al. 2002). Criticism has been presented especially concerning the two beneficial studies and their control groups, in which the ventilatory strategy

was not based on the best practise at the time. Tidal volumes, as well as inspiratory pressures, were higher than used routinely and showed significant increase after the randomisation of the study. Thus, the meta-analysis presented that the positive results of two beneficial studies may have rather been a result of the injurious ventilatory strategy in the control groups rather than better treatment in the low tidal volume groups (Eichacker et al. 2002). The effect of large tidal volumes have, however, been shown to be clearly deleterious (Amato et al. 1998, ARDSNet 2000). Recommendations to use low tidal volumes in septic ALI/ARDS patients have been presented (Dellinger et al. 2008), mainly based on the largest multicentre study. The precise size of the tidal volume causing the least injury still remains open.

When ARDSNet researchers tested several lung-injury biomarkers (*vide supra*), plasma RAGE, a marker of epithelial injury, was shown to decrease significantly more in the low tidal volume group. This suggests that the better outcomes achieved with a lower tidal volume strategy, may be due to a lesser amount of epithelial injury (Calfee et al. 2008). The same kind of effect was seen in IL-6 levels, which decrease significantly in both groups from baseline to day 3, but lowered even more in the lower tidal volume group (Parsons et al. 2005a).

Given these observations in ALI and ARDS patients, the field of interest has extended to mechanically ventilated patients who present risk factors for ALI at baseline, but no pre-existing condition. In a retrospective analysis of 332 mechanically ventilated patients without ALI at baseline, tidal volume per PBW was found to be an independent predictor for the development of ALI (Gajic et al. 2004). Later, an observational cohort study comparing two subsequent groups of mechanically ventilated patients without ALI at baseline, showed a significant reduction in tidal volumes as well as in the incidence of ALI (Yilmaz et al. 2007). An intervention in this study, carried out between the patient cohorts, was an introduction of protocol to limit tidal volumes and restrict transfusion policy. The magnitude of the decrease in ALI incidence over time (from 28% in 2001 to 10% in 2005) as well as in tidal volumes (from 10.6 to 7.7. ml/kg PBW) is impressive, especially regarding the simplicity of the intervention. The study setting with historical controls, however, may confuse the results. In a more recent report, the results of earlier studies were confirmed in a prospective, randomized setting evaluating the effect of tidal volume on ALI development in 150 patients (Determann et al. 2010). ALI incidence was 13.5% in a conventional tidal volume group (10 ml/kg PBW) versus 2.6 % in a low tidal volume group (6 ml/kg PBW), and the study was stopped prematurely for safety reasons. In both groups, plasma IL-6 levels decreased over time, but the decrease was more pronounced in the low tidal volume group. This result suggests that ventilation with lower tidal volumes results in a lower level of systemic inflammation.

2.2.3. EPITHELIAL INJURY

Surfactant proteins A (SP-A) and D (SP-D) are glycoproteins that belong to a family of collectins. They are secreted by alveolar epithelial type II cells. SP-A is the most abundant protein in surfactant, acting in the regulation of surfactant homeostasis and in host defence (Levine AM 1998, Wright JR 1993). It has been shown to inhibit the apoptosis of alveolar epithelial type II cells *in vitro* (White et al. 2001), and SP-A concentrations have been reported to decrease in a BAL fluid of early ARDS patients (Greene et al. 1999). SP-D, on the other hand, has an effect on surfactant phospholipid homeostasis (Botas et al. 1998), and plays an important role in the regulation of lung inflammation (Greene et al. 1999) and innate immunity in the lung (Wright 1997). Both surfactant proteins have been evaluated in 38 invasively ventilated patients, and results suggested that low oedema fluid levels of SP-A and high plasma levels of SP-D may be associated with worse outcome (Cheng et al. 2003). Later, these proteins were studied in patients from a large multicentre study, which was originally designed to compare two ventilatory strategies (ARDSNet 2000). SP-A did not show association with any clinical outcome measure (Eisner et al. 2003). High plasma SP-D levels, on the contrary, were associated with increased mortality and morbidity, and showed attenuation with low tidal volume strategy.

Clara cell secretory protein 16 (CC16) is a major protein secreted by Clara cells, multifunctional epithelial cells that act in the repair process of damaged alveolar epithelium (Broeckaert et al. 2000). CC16 levels have been shown to increase after five hours of mechanical ventilation for elective surgery but tidal volume did not affect its levels (Determann et al. 2008). In a small multicentre study, serum CC16 levels were associated with an increased risk of death at 28 days, however the AUC for the prediction of death was low at 0.68 (Lesur et al. 2006).

Receptor for advanced glycation end-products (RAGE) is a multi-ligand receptor expressed mainly by lung epithelial type I cells (Uchida et al. 2006). Cells from other organs, including nervous system and vascular endothelium, can also produce RAGE, but it is most abundant in lung (Brett et al. 1993). In immunoelectron microscopy, RAGE has shown to be situated in the basal membrane of type I epithelial cells in rat lungs (Shirasawa et al. 2004). Its ligands include, among others, cytokine-like mediators like S100/calgranulins, and high-mobility group box-1, a nuclear protein related to cell necrosis. Ligand binding to RAGE leads to the activation of proinflammatory pathways (mediated by nuclear factor- κ B), suggesting that it plays a potential role in systemic inflammation (Chavakis et al. 2004). Indeed, RAGE knockout mice have been shown to be able to resist experimental septic shock (Liliensiek et al. 2004). In addition, an association with chronic inflammatory stages, including diabetic complications and amyloidosis, has been detected (Schmidt et al. 2001). RAGE has been reported to consist of three domains, including a soluble form of RAGE (sRAGE) that represents the

extracellular domain (Uchida et al. 2006). It is thought to emerge into alveolar oedema fluid and plasma by metalloproteinase cleavage (Zhang et al. 2008) or it may represent endogenously secreted RAGE variants (Cheng et al. 2005). In patients with ALI, high levels of sRAGE has been detected in pulmonary oedema fluid and plasma (Uchida et al. 2006). In contrast, in mechanically ventilated hydrostatic pulmonary oedema patients, RAGE was detected in oedema fluid, although at lower levels than in ALI patients, but not in plasma. This finding strengthened the hypothesis that lungs would be a predominant source of sRAGE and suggested that mechanical ventilation may cause slightly increased sRAGE levels in hydrostatic pulmonary oedema patients. In a multicentre study that evaluated sRAGE in 676 ALI/ARDS patients, increased levels associated strongly with a clinical severity of lung injury (Calfee et al. 2008). In the same study, baseline plasma sRAGE was an independent predictor of 180 day mortality, but only in the group of 12 ml/kg tidal volume. These results have provided clinical evidence for epithelial injury in the pathophysiology of ALI, as well as the possible mechanism by which the use of low tidal volumes may affect the outcome. In a more recent study, levels of sRAGE were significantly higher in ALI/ARDS patients with or without severe sepsis or septic shock when compared to septic patients without ALI/ARDS, which reinforces even further the likely role of lungs as a primary source of sRAGE (Jabaudon et al. 2011)

2.2.4. ENDOTHELIAL INJURY

Von Willebrand factor (vWf) is a large multimeric protein that is secreted by endothelial cells and to a smaller extent by platelets. It acts in primary hemostasis by bridging platelets to endothelium, and as a carrier protein for factor VIII. Elevated levels have been found in patients with ARF caused by different reasons (Carvalho et al. 1982). vWf levels 450% or greater have been shown to be associated to the development of ALI in patients with non-pulmonary sepsis (Rubin et al. 1990). Nonetheless, contradictory results regarding the prognostic property of increased vWf levels have been published (Moss et al. 1995). Differences in the definitions of ALI and ARDS, as well as in patient material may explain this. More recently, increased levels of vWf have shown to be a potential prognostic biomarker of endothelial injury (Ware et al. 2001a), which was later confirmed in a large multicentre study (Ware et al. 2004). The ventilatory strategy, thus low tidal volume (6 ml/kg) as compared to conventional tidal volume (12 ml/kg), did not affect vWf levels, suggesting that the effect of the lung protective ventilatory strategy is primarily based on the attenuation of the epithelial injury. The exact source of vWf in ALI remains unclear. Pulmonary endothelium seems the most obvious place of increased

release, but systemic endothelium has also been proposed. This hypothesis offers one potential explanation for a pathway of indirect pulmonary injury. In healthy volunteers, infusion of tumor necrosis factor- α increased the vWf concentrations and simultaneously reduced the endothelial staining in skin biopsy, suggesting release of vWf from systemic endothelium (McGill et al. 1998).

Intercellular adhesion molecule-1 (ICAM-1) is present in endothelial cells and leukocytes and is considered to be a marker of both endothelial and epithelial injury (Conner et al. 1999). The production of ICAM-1 is induced by several cytokines, including IL-1 and TNF- α , and can be suppressed by glucocorticoids (van de Stolpe et al. 1996). During inflammation and injury, it mediates leukocyte migration through endothelium (Hordijk 2006). The exact role of ICAM-1 in the epithelium is not clear, but it has been shown to accelerate the inflammatory reaction in injury by activating alveolar macrophages (Schmal et al. 1998). In contrast, inhibition of lymphocyte adhesion by soluble ICAM-1 has also been noticed, suggesting the possibility of anti-inflammatory activity in some circumstances (Shingu et al. 1994). In a single centre study, plasma ICAM-1 levels were found to be higher in ALI patients than in the control group of hydrostatic pulmonary oedema patients (Calfee et al. 2009). Although a large multicentre study has shown the plasma levels to be predictors of some outcome parameters (ventilator-free days, organ-failure free days), its value as a mortality predictor seems more uncertain (Calfee et al. 2009). In an earlier study, ICAM-1 was unable to predict either the development of ARDS or the mortality, but the patient number of this study was small for predictive purposes (Agouridakis et al. 2002).

2.2.5. SYSTEMIC INFLAMMATION

Cytokines are an essential part of host defense mechanisms, acting in both innate and adaptive immunity. In critically ill patients, TNF- α and interleukin (IL)-1 are the main proinflammatory cytokines, while IL-6 has anti- and proinflammatory actions, and IL-10 is solely anti-inflammatory (Oberholzer et al. 2000). Cytokines are secreted by mononuclear cells, mainly monocytes and macrophages, in response to microbial antigens and inflammation. Proinflammatory cytokines activate further macrophages and neutrophils, induce endothelial cell production of adhesion proteins (including ICAM-1), mediate neutrophil migration, and stimulate the synthesis of acute phase proteins (Riedemann et al. 2003). IL-10 downregulates many aspects in acute inflammatory process, including proinflammatory cytokine production of activated neutrophils and macrophages (Opal et al. 2000).

Increased levels of cytokines have been detected in patients with ARDS and persistently increased levels in plasma (Meduri et al. 1995) and BAL (Goodman et al. 1996) have been shown to associate with poor outcome. In a multicentre

trial, levels of IL-6, IL-8 (another proinflammatory interleukin), and IL-10 were all significantly higher in non-survivors than survivors at baseline and at day 3 (Parsons et al. 2005a). The lung protective ventilation strategy was associated with a greater decrease in IL-6 and IL-8 concentrations, suggesting that injurious ventilation sustains the inflammatory reaction in lungs. More recently, in a small prospective study where several biomarkers were tested in 50 ALI patients, levels of IL-8, but not IL-6, differed between survivors and non-survivors (McClintock et al. 2008). These somewhat conflicting results are most probably due to inadequate sample size in the latter study.

TNF- α is known to be an important mediator of ALI (Parsons et al. 1992, Tracey et al. 1988). In an animal study, a recombinant human soluble TNF receptor (TNFR) fusion protein, which is able to bind to TNF- α , thus inhibit its activity, attenuated the development of ALI (Wolthuis et al. 2009). Clinical studies have not, however, been able to confirm the association with TNF- α levels and clinical outcome in ALI patients or patients with risk factors for ALI (Park et al. 2001, Pittet et al. 1997). It has been suggested that this may be due to soluble TNF receptors I and II (sTNFR I and II). TNFR I and II are released widely from variety of cells, but epithelial cells mainly express TNFR I. Inflammatory stimuli are reported to cause shedding of these receptors from cell surfaces (Mackay et al. 1993). sTNFRs are able to bind TNF and thus, by competing with the cell surface receptors, downregulate TNF activity. Elevated levels of sTNFRs have been reported after trauma (Hensler 2002) and coronary bypass operation (ElBarbary MKKS 2002). Based on these findings, Parsons and colleagues evaluated the levels of TNFR I and II in ALI patients first in a single centre study and later in a multicentre trial (Parsons et al. 2005b). In a single centre study, levels of sTNFR I and II in patients with risk factors for ALI were not able to predict the development of ALI. In a multicentre trial of 562 ALI/ARDS patients randomized to 6 ml/kg versus 12 ml/kg ventilatory strategy, sTNFR I and II were independent predictors of mortality. sTNFR I, but not sTNFR II, was attenuated by the 6 ml/kg ventilation strategy. Additionally, sTNFR I was reported to be released from alveolar epithelial cells in an experimental substudy, findings supporting the epithelial origin of sTNFR I. It is of interest to note that TNF- α levels, measured from a subgroup of 377 patients, were detectable in only 34 (9%) patients and were comparable in survivors and non-survivors. These results suggest that sTNFRs are markers of inflammation, and perhaps also of epithelial injury in ALI. Additionally, TNF- α does not appear to be an appropriate biomarker.

2.2.6. COAGULATION

Inflammation and coagulation cascades co-operate closely (Levi et al. 1999). The early mediators of the inflammatory process, TNF- α , IL-1 and IL-6, are known to

activate coagulation via the tissue factor (TF) pathway (or extrinsic pathway), and also to stimulate the release of plasminogen activator inhibitors (PAI), resulting in decreased fibrinolysis. The activation of coagulation proceeds primarily via an extrinsic pathway where TF binds to factor VII (fVII) in sepsis (Biemond et al. 1995), and in ARDS and ventilator associated pneumonia (Idell et al. 1987a, Schultz et al. 2004). The activated TF/fVIIa complex further activates fX to fXa leading to alveolar thrombin, and eventually to fibrin formation.

In addition to its hemostatic effect, thrombin has a key role in pulmonary fibroproliferation, which is mediated by protease activated receptor -1 (PAR-1), a major thrombin receptor (Howell et al. 2002). The expression of PAR-1 on cell surfaces can be upregulated by proinflammatory and profibrotic mediators (Sokolova et al. 2007), and it is proposed to play a central role in coordinating the interactions between coagulation, inflammation, and fibrosis in lung injury (Chambers 2008). In addition to thrombin, fXa is also able to activate PAR-1 and induce fibrotic responses in the mouse lung (Blanc-Brude et al. 2005, Scotton et al. 2009). In an animal study, PAR-1-deficient mice were protected against pulmonary fibrosis (Howell et al. 2005). The protective action of PAR-1 deficiency was accompanied by the reduction of proinflammatory and profibrotic mediators, TGF- β 1 among others, known to be induced by PAR-1. In addition, direct inhibition of thrombin has been shown to reduce lung collagen accumulation in an experimental study (Howell et al. 2001). Indeed, reports of the actions of PAR-1 have been limited to experimental settings.

The natural inhibitor of coagulation, namely activated protein C (APC) has an essential role in the regulation of coagulation. Its role in human sepsis has been studied extensively, and a growing body of evidence suggests that it is also involved in ALI. APC has antithrombotic, profibrinolytic, and anti-inflammatory properties. Its concentrations in plasma and BAL fluid were reported to be significantly lower than normal in 45 patients with ALI or ARDS, and lower levels have been associated with increased hospital mortality, independent of sepsis (Ware et al. 2003). Later, the results were confirmed in a multicentre study of 779 patients that showed protein C to be an independent predictor of hospital mortality (Ware et al. 2007). Additionally, plasminogen activator inhibitor-1 (PAI-1) concentrations were evaluated as markers of fibrinolysis, which was noticed to decrease. PAI-1 also was an independent predictor of death, but more importantly, a synergistic interaction between protein C and PAI-1 was observed, where low protein C and high PAI-1 levels coincided. The data suggests that when either fibrinolysis or coagulation impairment exists, the other is able to compensate partly, the situation being contrary if both are impaired. More recently, in another study with 50 patients, levels of other markers of inflammation, coagulation, and fibrinolysis were also evaluated in addition to protein C. All markers (IL-8, sICAM-1, protein C, thrombomodulin, and PAI-1) except IL-6, differed significantly between survivors and non-survivors, confirming

the broad abnormalities in these pathways in ALI (McClintock et al. 2008).

A decade ago, a multicentre RCT evaluating recombinant human APC versus placebo in severe sepsis, showed a significant reduction in overall mortality (Bernard et al. 2001). Additionally, those patients with respiratory dysfunction and treated with APC, recovered faster. Thus far, APC has remained the only specific treatment in severe sepsis. However, the manufacturer has just withdrawn the medicine from the worldwide market after the preliminary results of a large multicentre study which did not show any difference in the mortality between APC and placebo in septic shock patients (Eli Lilly, press release, October 2011). Based on earlier findings and the pathophysiological rationale, a multicentre phase II study evaluating APC and placebo in non-septic ALI patients was performed; however, the study was stopped early because of no effect in the treatment group (Liu et al. 2008).

PAI-1 inhibits plasminogen activator, the action leading to decreased fibrinolysis. PAI-1 release is increased by proinflammatory mediators, TNF- α and IL-1. Elevated levels of PAI-1 in plasma and especially in BAL fluid has been reported in patients with ALI in comparison to hydrostatic pulmonary oedema, suggesting inhibition of fibrinolytic activity in ALI (Prabhakaran et al. 2003). The levels of plasma PAI-1 were significantly higher in non-survivors than survivors, a result that was later confirmed in a multicentre study, which showed PAI-1 to independently predict mortality (Ware et al. 2007), as mentioned earlier.

Nitric oxide (NO) is a marker of oxidative injury (Baylis et al. 1998). It reacts with superoxide ion and forms peroxynitrite, a highly reactive intermediate. It nitrates and oxidizes and, thus, inactivates proteins such as SP-A, and inhibits their functions (Ehrhart et al. 2000). Due to the short half life of peroxynitrite, nitric oxide species (NO, nitrate and nitrite) are used to measure its activity. Studies on animal lung injury have suggested that in lung injury, BAL fluid levels of nitric oxide species are higher than in controls (Enkhbaatar et al. 2003, Frank et al. 2003). A single center clinical study confirmed these results in humans, and also suggested that the higher NO levels in BAL fluid associate with mortality (Sittipunt et al. 2001). In a multicentre study, however, the results were contrary, where higher levels of urine NO were associated with better outcome by means of reduced mortality and fewer days in a ventilator and with organ failure (McClintock et al. 2007). The role of NO as a biomarker of ALI, thus, remains uncertain, and requires further studies.

Desmosine, a breakdown product of elastin, has been studied in different chronic pulmonary diseases, including COPD, cystic fibrosis, and tobacco use (Bode et al. 2000, Cocci et al. 2002). Desmosine is excreted to urine, and is a selective marker of elastin breakdown. Urine desmosine has shown promise as a marker of mortality in a multicentre study, but ventilatory strategy did not associate with desmosine levels (McClintock et al. 2006). The prognostic role of urine desmosine in ALI remains unconfirmed.

2.2.7. APOPTOSIS

Apoptosis plays an important role in the maintenance of normal tissue structure. Apoptosis, a controlled cell death, can be activated in several ways, as genetic “programmed cell death”, via an external pathway, and through receptor-ligand interactions (Perl et al. 2005a). As a result of cell death, DNA is fragmented and can be found in the circulation. Concerning the pathogenesis of ALI, apoptosis may play role at several stages.

The apoptotic death of alveolar epithelial cells has been suggested to play a major role in the development of ALI (Matute-Bello et al. 2001a, Matute-Bello et al. 2001b). The mechanisms of epithelial cell apoptosis are not well understood, but the role of an “extrinsic” or death receptor pathway is supported by several studies (Matute-Bello et al. 1999, Perl et al. 2005b). This pathway is mediated by the cell surface receptor Fas (also known as CD95) and its natural ligand, Fas-ligand, and can be initiated in response to different soluble mediators. The expression of Fas-receptor on the surfaces of alveolar cells is increased by the stimulation of inflammatory mediators (Kitamura et al. 2001). Fas-ligand can exist in soluble and membrane bound form, and both are able to activate Fas-receptor and start the apoptotic pathway. In an animal study, rabbits ventilated with high tidal volume, presented increased apoptosis, measured by plasma levels of Fas-ligand (Imai et al. 2003). Caspases, a family of proteolytic enzymes, are thought to have an essential role in intracellular pathways resulting in DNA cleavage as a final step (Perl et al. 2005a). Fas-ligand has shown to be present in a biologically active form in BAL fluid from early ARDS patients, and high concentrations associated with worse outcome (Matute-Bello et al. 1999). Angiotensin (AT) II has been shown to induce epithelial cell apoptosis *in vitro* via Fas/Fas ligand –pathway (Wang et al. 1999), and increased amounts of ATII have been detected in BAL fluid in ARDS (Idell et al. 1987b).

Neutrophilic apoptosis is another important mechanism by which apoptosis has been shown to be involved with ALI (Figure 1). Neutrophils are important cells in the first line of host defence against pathogens. They migrate through the injured endothelium to the alveolar space, where they secrete proteases, leukotriens, oxidants, and platelet activating factor (PAF), thus, further enhance the inflammatory reaction. After this, neutrophils apoptose and are cleared from the injury site by phagocytotic cells like macrophages. In a rat lung injury model, neutrophil apoptosis has been reported to be inhibited in the early moments after experimental lung injury, however recovery was evident at 24 hours with a clear clinical and histological picture of early resolution of injury (Hussain et al. 1998). Another rat study has shown an enhancement of neutrophil apoptosis to associate with decreased mortality and lung injury (Sookhai et al. 2002). Also in animal studies, the stimulation of neutrophil release from bone marrow with

human granulocyte colony-stimulating factor (G-CSF) (filgrastim) has been shown to worsen lung function in ALI (King et al. 1995) and the outcome in pneumonia (Held et al. 1998). On the contrary, in human studies these detrimental effects of G-CSF have not been observed in patients with community acquired pneumonia (Nelson et al. 1998) or in mechanically ventilated ICU patients (Pettila et al. 2000). Clearance failure of apoptotic neutrophils is associated with increased inflammation and decreased survival in the CD44 deficient mouse model of acute lung injury (Teder et al. 2002). The phagocytic activity of apoptotic neutrophils attenuates the production of proinflammatory cytokines and stimulates the production of anti-inflammatory mediators, thus, favouring resolution of inflammation. In contrast, phagocytotic clearance of necrotic cells is related to the release of proinflammatory cytokines and thus, potentially inducing tissue injury (Fadok et al. 1998).

2.2.7.1 Plasma cell-free DNA

The detection of fetal cell-free DNA in maternal blood has been used in the evaluation of prenatal conditions (Wright et al. 2009). It has also been studied widely in different malignancies, but its role in that area remains uncertain (van der Vaart et al. 2010). Studies have also evaluated cell-free DNA in different states of acute illness, including abdominal pain, chest pain, stroke, pancreatitis, trauma, ischemic cardiomyopathy, and resuscitated patients (Arnalich et al. 2010b, Gornik et al. 2009, Lo et al. 2000, Rainer et al. 2008, Rainer et al. 2006, Rainer et al. 2003, Zaravinos et al. 2011). Few studies reporting critically ill patients have been published (Martins et al. 2000, Rhodes et al. 2006, Saukkonen et al. 2008, Saukkonen et al. 2007, Wijeratne et al. 2004). Table 5 summarizes studies, which have evaluated the prognostic value in ICU patients.

The levels of cell-free DNA in plasma can reflect different phenomenon. In ALI, the apoptosis of neutrophils and lung epithelial cells most likely plays an important role, as reviewed above. Cell necrosis and apoptosis are well known origins of cell-free DNA in blood (Jung et al. 2010). In healthy individuals apoptosis is considered a primary mechanism for DNA occurrence in blood (Suzuki et al. 2008). Active release of DNA fragments from living cells has also been described. DNA can be extracted from both serum and plasma, however plasma samples are more reliable because serum samples can contain DNA originating from *in vitro* lysis of leukocytes (Lee et al. 2001).

There is a lack of knowledge regarding the elimination process of cell-free DNA from blood. It is known that a half-life of four to 30 minutes is evident for the clearance of fetal DNA from maternal blood after delivery (Lo et al. 1999). Renal failure does not seem to affect the elimination since no differences were detected

in the concentrations of plasma cell-free DNA between healthy volunteers and patients with chronic renal failure (Garcia Moreira et al. 2006). Decreased activity of deoxyribonuclease as a reason for high cell-free DNA concentration has been reported (Cherepanova et al. 2008), but the activity of plasma nucleases in the elimination process is considered insignificant (Jung et al. 2010).

Table 5. Studies evaluating prognostic value of plasma cell-free DNA in critically-ill patients

	Number	Patients included	best cut-off (GE/ml)	AUC (mortality)	Sensitivity, specificity for hospital mortality
Wijeratne 2004	96	Consecutive patients admitted in ITU	6100	0.889 (ITU)	sensitivity 85% specificity 80%
Rhodes 2006	52	Unselected ICU admissions	19 000	0.84 (ICU) * 0.79 (hospital)	sensitivity 81% * specificity 81% *
Saukkonen 2007	228	Unselected ICU admissions	NA	NA	NA
Saukkonen 2008	255	Severe sepsis or septic shock	12 000	0.71 (ICU) * 0.61 (hospital)	sensitivity 67% * specificity 67% *

AUC, area under receiver operating curve; GE, genome equivalent; ICU, intensive care unit; ITU, intensive treatment unit; NA, not available

* sensitivity and specificity for ICU mortality

2.2.8. FIBROGENESIS

The development of lung injury has been previously considered to take place in subsequent steps where the accumulation of collagen starts only after five to seven days. A growing collection of data, however, supports the likelihood that the process of fibrogenesis starts early after the insult (Chesnutt et al. 1997, Entzian et al. 1990, Liebler et al. 1998). Indeed, it has been shown that the collagen messenger RNA expression is upregulated immediately after cardiopulmonary bypass, an insult known to predispose to ARDS (Deheinzelin et al. 1997).

The cells responsible for collagen production are myofibroblasts that arise from the activation of tissue fibroblasts, bone-marrow-derived fibroblasts, and fibroblasts experiencing epithelial-to-mesenchymal transition (Kisseleva et al. 2008). Fibrin is thought to act as a provisional matrix, on which the proliferation and collagen production of myofibroblasts takes place (Pohl et al. 1979). A proinflammatory mediator, TGF- β 1, has a major role in the activation of fibroblasts in wound healing

as well as in idiopathic pulmonary fibrosis (Scotton et al. 2007, Singer et al. 1999). A latent form of TGF- β 1 is known to be activated by numerous stimuli, including metalloproteinases, plasmin, and reactive oxygen species, all acting in the process of acute inflammation (Mu et al. 2002). The role of TGF- β 1 in ARDS has been reported for the first time in the study of 13 early ARDS patients, whom showed elevated TGF- β 1 activity in BAL fluid (Fahy et al. 2003).

2.2.8.1. Markers of collagen metabolism

Collagen types I and III are the dominating proteins of extracellular matrix and are produced in response to tissue injury. The balance of collagen synthesis and degradation is important in order to maintain tissue integrity. In fibrosis, excessive collagen accumulation in tissues leads to organ failure (Adler et al. 1989, Eddy 1995, Weber 1989).

Collagen III, a reticular fibre, is synthesised quickly by young fibroblasts and is common in granulation tissue. The synthesis of collagen I, the most abundant collagen in human body, takes place later in the course of fibrogenesis and its structure is more stable and resistant, confirming the breaking strength of tissue. Endoplasmic reticulum of myofibroblasts synthesises α -chains, which subsequently combine, in a zipper-like manner, to form a triple helix structure of procollagen molecule. After secretion to the extracellular space, pro-collagen molecules first go through proteolytical cleavage of carboxy(C)- and amino(N)-terminal ends, and then connect with each other to form insoluble collagen fibrils. These fibrils arrange further to form collagen fibres (Myllyharju et al. 2004). C- and N-terminal ends of procollagen, by-products of collagen synthesis, are produced in equimolar amounts to collagen and, thus, reflect the rate of collagen synthesis. Human studies have shown increased serum levels of procollagen III in trauma patients (Waydhas et al. 1993). In ALI and ARDS patients, procollagen accumulation in BAL fluid has been reported, indicating increased collagen synthesis in lungs (Armstrong et al. 1999, Chesnutt et al. 1997, Clark et al. 1995).

Studies on ICTP, the degradation product of collagen type I, cover mainly diseases of skeleton, a main source of collagen breakdown products (Heider et al. 2006). In critically ill patients, elevations of ICTP, has been measured in two studies in patients with severe sepsis (Gaddnas et al. 2009, Wenisch et al. 1996).

Concentrations of all collagen metabolites measured from serum reflect the metabolism of the whole body. Studies have shown that in lungs (Marshall et al. 2000) increased collagen synthesis is obvious, but for example in undamaged skin of septic patients, the synthesis is depressed (Gaddnas et al. 2010).

2.2.9. RESOLUTION

Apoptosis of myofibroblasts responsible for producing collagens, is a key step in cessation of the repair process (Weber 1997). Following apoptosis of myofibroblasts, and the subsequent reduction in TGF- β 1 secretion, the process of repair abates (Hussain et al. 1998).

The recovery of alveolar fluid clearance has been suggested to have a major role in the resolution of ALI (Ware et al. 2001b). Traditionally, alveolar epithelial type II cells have been considered to be responsible for the active Na⁺ and Cl⁻ ion transport, which is followed by inactive water shift through type I epithelial cells. Recently, epithelial type I cells have been shown to play a major role in ion transport and its following fluid shift (Johnson et al. 2006). The role of special water channels, “aquaporins”, in the alveolar fluid clearance has been challenged (Verkman 2007). β -adrenergic agonists are known to stimulate sodium transport and, thus, increase alveolar fluid clearance, an observation from isolated human lung (Sakuma et al. 1994) and from an animal study (McAuley et al. 2004). More recently, the effect of intravenous infusion of salbutamol has been studied in a single centre RCT including 40 patients with ALI/ARDS. Patients in the salbutamol group had significantly lower lung water and plateau pressure at day seven when compared to the placebo group (Perkins et al. 2006). In contrast, amiloride and propranolol decrease sodium transport.

Type II alveolar epithelial cells (AEC) are responsible for the production of surfactant, and act in the first line of host defence, but are essential also in the repair process. Type II AECs are mesenchymal cells with a pluripotent character, and they are able to differentiate to type I epithelial cells and restore the damaged epithelium (Mason 2006). The apoptosis is thought to play a role, in addition to myofibroblasts, in the clearance of neutrophils and the excess type II AECs.

In conclusion, many biomarkers have shown distinct abnormalities in patients with ALI, but none studied so far can be considered useful in clinical practise. The knowledge of the pathophysiological pathways, however, has increased enormously, and will hopefully yield new treatments for ALI in the future.

2.3. MEASURING OUTCOME

Outcome can be measured with multiple parameters. Short-term outcome is mandatory for long-term outcome, but long-term outcome is critically important. ICU and hospital mortalities are commonly used as short-term outcome parameters, but recently longer periods of survival have been used increasingly as outcome measures (Bellomo et al. 2009, Finfer et al. 2009). Even five year survival rates after ARF have been reported (Garland et al. 2004). The need for mechanical

ventilation has been predicted to increase with the ageing population (Needham et al. 2005). Along with the increased costs of intensive care, associated with mechanical ventilation, the importance of studies of long-term outcome is highlighted. Reporting health related quality of life after critical illness has gained increasing interest. Few studies reporting quality adjusted life years, or cost-effectiveness, have been published (Angus et al. 2001, Linko et al. 2010). Similar studies are warranted, since long-term morbidity and disability after ARDS has been reported (Herridge et al. 2003).

Physiological parameters are frequently used in clinical studies, but they have proven to be poor surrogates as long-term outcome predictors in ALI. In a large multicentre study evaluating the use of inhaled nitric oxide, improved oxygenation was not translated to better outcome (Lundin et al. 1999). Similar observations of improved oxygenation without outcome benefit include studies on exogenous surfactant (Spragg et al. 2004) and prone position (Gattinoni et al. 2001). In contrast, in the ARDSNet study of tidal volumes, worse oxygenation and higher carbon-dioxide partial pressure was seen in the acute stage of treatment, but finally, all outcome parameters were significantly better in the group of lower tidal volume (6 ml/kg) in comparison with traditional tidal volume (12 ml/kg) (ARDSNet 2000).

3 AIMS OF THE STUDY

The objective of this study was to evaluate the incidence, the treatment, and the outcome of acute respiratory failure in Finland, with special interest in the use of biomarkers in the prediction of outcome and the multi organ dysfunction. The aims in detail were as follows:

1. To evaluate the incidence, the treatment, and the outcome of acute respiratory failure (ARF) in patients who were treated in Finnish intensive care units (I).
2. To investigate the levels of aminoterminal pro-brain natriuretic peptide (NT-pro-BNP) (II) and plasma cell-free DNA (III) in ARF patients, with special interest in their prognostic value.
3. To study the collagen synthesis and degradation and their association with multiple organ dysfunction in ARF patients with prolonged hospital stay. (IV)

4 PATIENTS AND METHODS

4.1. PATIENTS

This study consists of 958 adult patients in 25 ICUs who were treated with either invasive or non-invasive ventilatory support for at least six hours. All 958 patients took part in Study I. In studies II-IV patients with laboratory samples drawn for analysis of NT-pro-BNP (Study II), plasma cell-free DNA (Study III), and markers of collagen synthesis and degradation (Study IV) were evaluated. Patients included in studies I-IV are presented in Figure 2. The characteristics of patients included in Studies I-IV are presented in Table 6.

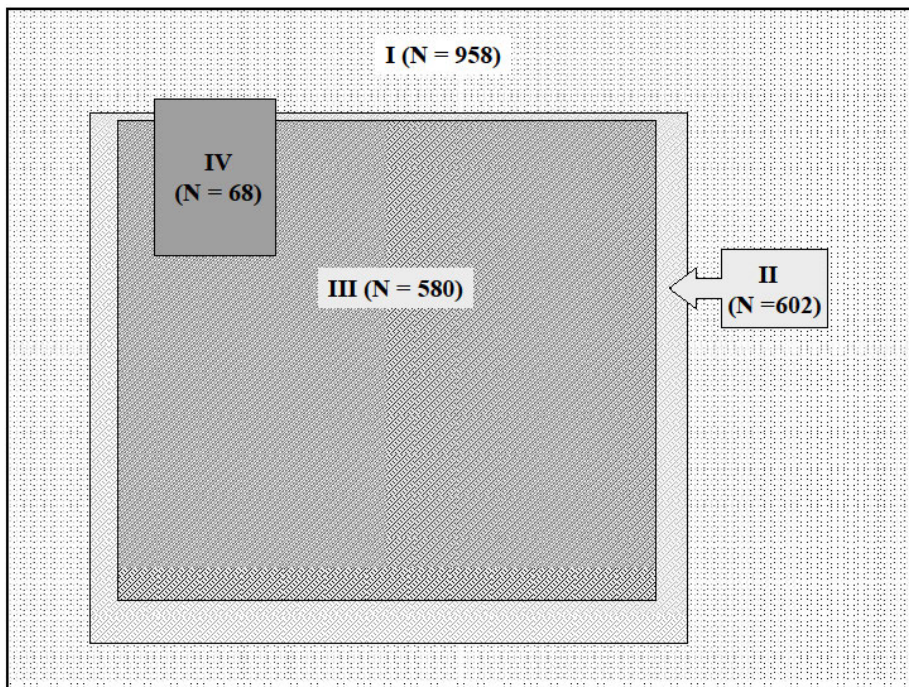


Figure 2. Patients included in studies I-IV

Table 6. Characteristics of patients included in studies I-IV.

	Study I	Study II	Study III	Study IV
Patient number	958	602	580	68
Age	63 (51 - 74)	64 (53 - 75)	65 (53 - 75)	60 (50 - 70)
Gender, male (%)	637 (67)	398 (66)	382 (66)	50 (74)
Emergency	824 (86)	502 (83)	481 (83)	63 (93)
Operative	375 (39)	232 (39)	229 (40)	18 (27)
APACHE II	23 (17 - 29)	23 (17 - 30)	23 (17 - 30)	23 (18 - 29)
SAPS II	43 (31 - 55)	42 (29 - 55)	42 (30 - 55)	44 (34 - 54)
SOFA at 24 h	8 (6 - 10)	8 (5 - 10)	8 (5 - 10)	9 (5 - 10)
Worst PaO ₂ /FiO ₂ (first 3 days), mmHg	171 (118 - 234)	176 (123 - 236)	176 (124 - 236)	150 (110 - 209)
Ventilatory support time, days	2 (1 - 4)	2 (1 - 4)	2 (1 - 4)	8 (4 - 17)
ICU length of stay, days	3 (2 - 7)	3 (2 - 7)	3 (2 - 7)	11 (6 - 23)
Hospital length of stay, day	11 (6 - 21)	12 (6 - 21)	12 (6 - 21)	38 (26 - 52)
ICU mortality, n (%)	120 (13)	63 (11)	61 (11)	1 (2)
Hospital mortality, n (%)	230 (24)	138 (23)	134 (23)	6 (9)
90-day mortality, n (%)	295 (31)	174 (29)	169 (29)	8 (12)

Data are presented as median with interquartile range (IQR) or number and percentage (%).

4.2. STUDY DESIGNS

This study was a prospective cohort study conducted in Finnish intensive care units in 2007. All 27 ICUs were invited to participate in the study and 25 units consented. During an eight week period from April 16th to June 10th, all consecutive adult (≥ 16 years) patients admitted to participating ICUs were screened for acute respiratory failure. Blood samples at four time points, at study admission (day 0) and at days 2, 7, and 21, were drawn if the patient was still hospitalised. The ethical committee of the Surgical Department in Helsinki University Central Hospital approved the study. Written informed consent was not required for data registration, but only for blood sampling.

Study I

Study I is a description of the whole cohort. All periods of respiratory support were screened and the reason for support lasting less than six hours was recorded. The exclusion criteria were being aged under 16 years and the need for permanent ventilatory assistance. The patients' social security number was used for mortality

assessment and in the combining of the data of the related periods in the separate ICUs. Only the first period of ARF was counted for incidence calculations. The adult population (≥ 16 years) count in Finland at the end of year 2006 was obtained from Statistics Finland (<http://www.stat.fi>). The adult population of hospital districts of two ICUs not participating in the study was attained from the Association of Finnish Local and Regional Authorities (<http://kunnat.net>) and subtracted. In the data processing and analysis, a unique ID number was used.

Study II

Study II is a substudy of study I. The primary aim was to evaluate the prognostic value of NT-pro-BNP according to 90-day mortality in an unselected cohort of acute respiratory failure patients. Samples were analysed in 602 patients at baseline (day 0) and in 505 patients on day 2.

Study III

In study III, also a substudy of study I, the levels of plasma cell-free DNA and their potential in the prediction of outcome in ARF patients were investigated. Plasma samples were drawn from 580 patients at baseline (day 0) and in 489 patients on day 2.

Study IV

In study IV, we aimed to evaluate the change in the markers of collagen metabolism over time. Thus, only patients with serial blood samples from at least three time points (on days 0 and/or 2, 7 and 21) were included in this study. The association of collagen metabolism and multi organ dysfunction was also investigated. Patients were divided into groups according to the presence of MOD during the first week. A patient was defined to have MOD if the organ specific SOFA score of at least two organs was 3 or 4 on at least one day (Vincent et al. 2002). PINP and PIIINP were analysed as markers of collagen synthesis. ICTP was analysed as a marker of collagen I degradation. The ratio of PIIINP and PINP (PIIINP/PINP) was used to describe the relationship of the synthesis of two collagens with different temporal evolution. The ratio of PINP and ICTP (PINP/ICTP) was used to describe the relationship of type I collagen synthesis and degradation.

4.3. CLINICAL DATA

All 25 participating ICUs belonged to the Finnish Intensive Care Quality Consortium (Intensium®), which collects intensive-care data for benchmarking purposes. Data collection was accomplished with the clinical report form (CRF), which acted as a supplement to the routine consortium dataset. The routine consortium dataset includes the reason for ICU admission, outcome measures, and severity scorings [Acute Physiology and Chronic Health evaluation (APACHE) II (Knaus et al. 1985), Simplified Acute Physiology Score (SAPS) II (Le Gall et al. 1993) and Sequential Organ Failure Assessment (SOFA) score (Vincent et al. 1996)]. The CRF data included patient demographics, underlying risk factors for ARF, physiological and ventilatory data, and medications. The survival data were obtained from Statistics Finland (www.stat.fi). The CRF data were collected at study admission, once daily during the intensive care, and at discharge from the ICU. An internet-based interface was utilized to enter the routinely-collected data, and the CRF data was attached as an additional file. All of the data was stored in the ICU quality database.

4.4. MEASUREMENTS OF BIOMARKERS

After written consent from the patient or the next of kin, blood samples were drawn from an arterial line. A 10 ml plasma sample collected to a heparin tube, in addition to a 10 ml serum sample collected to a regular tube, was obtained at days 0 (study admission), 2, 7, and 21, if patient was still hospitalised. Samples were centrifuged 3100 rpm (~ 1500 g) for 15 minutes as soon as possible, after which plasma and serum were stored in each hospital at -20 °C or lower. After the study end, all samples were collected to Helsinki University Central Hospital and stored at -80 °C before subsequent analyses.

4.4.1 NT-PRO-BNP

Plasma NT-pro-BNP concentrations were determined after the first thaw using a commercial immunoassay (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany) on an Elecsys 2010 automatic analyzer (Roche Diagnostics, Mannheim, Germany). Two-fold dilution was accomplished if the result was above upper range (35 000 pg/ml). If the result was above 70 000 pg/ml, the further dilution was abandoned due to larger deviations in values after dilution and minor information achieved. All laboratory analyses were performed in a single accredited laboratory (Helsinki University Central Hospital Laboratory, HUSLAB).

4.4.2 PLASMA CELL-FREE DNA

The extraction of DNA and the quantification of plasma cell-free DNA was performed according to a method described earlier (Saukkonen et al. 2008, Saukkonen et al. 2007). Plasma samples were first centrifuged at 16 000 g for 10 min to remove any residual cells (Swinkels et al. 2003). DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the "blood and body fluid protocol". The measurement of plasma cell-free DNA was accomplished by real-time quantitative PCR assay for the β -globin gene. A 10-fold serial dilution of human genomic DNA (Roche, Mannheim, Germany) was used as a standard curve in the PCR assay. Results are expressed as genome equivalents (GE)/ml; 1 GE equals 6.6 picograms of DNA.

4.4.3 MARKERS OF COLLAGEN METABOLISM

Analyses of serum PINP, PIIINP, and ICTP were achieved by radioimmunological assays (Orion Diagnostica, Espoo, Finland). Reference values for PIIINP are 1.7 - 4.2 $\mu\text{g/L}$, for PINP 19 - 84 $\mu\text{g/L}$ (women) and 20 - 76 $\mu\text{g/L}$ (male), and for ICTP 1.6 - 4.6 $\mu\text{g/L}$ (Risteli et al. 2002). For serum intact PINP assay, the inter- and intra-assay coefficients of variation (CV) were between 3.1 and 9.3% for values within the reference intervals (Melkko et al. 1996). For serum PIIINP assay, inter- and intra-assay CVs were from 3.0 to 7.2% for values ranging from 2.7 to 12.2 $\mu\text{g/L}$. The CVs of the ICTP method ranged from 3 to 8% for a wide range of concentrations (Risteli et al. 1993)

4.5. DISEASE SEVERITY SCORINGS AND DEFINITIONS

The severity of disease at baseline was assessed by APACHE II (Knaus et al. 1985) and SAPS II (Le Gall et al. 1993) scores, which were calculated after 24 hours in the ICU. Sequential organ failure assessment (SOFA) score was calculated daily to define organ dysfunctions (Vincent et al. 1996). Multiple organ dysfunction (MOD) was defined as a SOFA score of 3-4 for at least two organs at the same time (Vincent et al. 2002) (IV). Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) were defined according to AECC consensus criteria (Bernard et al. 1994). The infection status was defined according to APACHE III, ICD-10 diagnoses, and underlying risk factors (pneumonia or sepsis during preceding 48 hours) (III).

4.6. OUTCOME

The mortality at 90-days was reported as long-term outcome measure (I-III). As short-term outcome parameters, ICU and hospital mortalities were reported (I-IV).

4.7. STATISTICAL METHODS

Data are presented in absolute numbers and percentages, in medians with interquartile ranges (25th and 75th percentiles, IQR), or means and standards deviations (SD) as appropriate.

Statistical significance. A p-value < 0.05 was considered significant in all analysis (I-IV). An exception for this is where the plasma cell-free DNA levels were compared between different strata of oxygenation. In this circumstance, Kruskal-Wallis test was used, and Mann-Whitney test in a post-hoc analysis, where $p < 0.01$ was considered significant due to multiple comparisons (III).

Fisher's exact test was used for comparisons of categorical data between groups (I, IV)

Kaplan-Meier survival curves of 90-day mortality were constructed according to quartiles of baseline NT-pro-BNP (II) and the best cut-off point of baseline plasma cell-free DNA (III). **Log rank test** was used for comparisons of different strata.

Kruskal-Wallis test was used for comparisons of multiple groups of continuous data (II-III)

Linear mixed model approach was used for the analyses of repeated measurements of fibrosis markers (IV).

Multivariate logistic regression analysis was used to evaluate the independent effect of variables on outcome (I-III).

Mann-Whitney U-test was used in the comparisons of continuous data between independent groups. It was used to compare demographic data (I-IV) and biomarker data (II and III).

Pearson's χ^2 - test was used in the comparisons of categorical data (IV).

Receiver operating characteristics (ROC) curve analysis was used to evaluate the prognostic value NT-pro-BNP and plasma cell-free DNA. The best cut-off values were identified by Youden method (Youden 1950), and the sensitivity, specificity, positive likelihood ratio (II and III), and positive and negative predictive values (II) for baseline NT-pro-BNP and cell-free DNA were calculated for it.

The area under ROC curve (AUC) was calculated with 95% confidential intervals (II, III).

Student's t-test was used for the comparisons of normally distributed continuous variables (IV).

5 RESULTS

5.1. THE INCIDENCE OF ARF, ALI AND ARDS IN FINLAND

During an eight week study period, 2670 patients were admitted to ICUs participating the study, and ventilatory support was accomplished in 1319 (49.4%) of admissions. In 273 (20.7%) of ventilatory support periods, treatment continued for less than six hours. After exclusion of these short treatment periods, multiple admissions, foreign citizens, and periods with incomplete data, there were 958 patients, who were treated for more than six hours with either invasive or non-invasive ventilatory support. The incidence of ARF, estimated from the incidence in the study period, was 149.5 / 100 000 inhabitants per one year. The incidence of ARF patients with ventilatory support for more than 24 hours was 102.7 / 100 000 per year. Of 958 patients, all criteria of ALI were fulfilled in 68 patients and those of ARDS in 32 patients. The incidence of ALI and ARDS defined by AECC criteria were 10.6 and 5.0 / 100 000 per year.

5.2. VENTILATORY TREATMENT

The majority of patients (775 of 958, 81%) were invasively ventilated from the start. During the treatment, 78 more patients were intubated, and 105 (11%) were treated only with non-invasive methods. At baseline, pressure controlled modes were used in 43% and volume controlled in 47% of cases. Modes allowing spontaneous triggering were used in 81%, while entirely controlled modes only in 9%.

The median (IQR) tidal volume at baseline was 7.4 (6.2 – 8.7) (ml/kg ABW) and 8.7 (7.6 – 9.9) (ml/kg PBW) in non-ALI/ARDS patients, and 7.5 (6.3 – 9.0) (ml/kg ABW) and 8.6 (7.3 – 9.9) (ml/kg PBW) in ALI/ARDS patients, $p = \text{NS}$. Plateau pressures in non-ALI/ARDS and ALI/ARDS patients were 19 (16 – 23) cmH_2O and 23 (18 – 27) cmH_2O , respectively, $p < 0.05$. The median PEEP level was 6 (5 – 8) cmH_2O in non-ALI/ARDS group and 8 (6 – 10) cmH_2O in ALI/ARDS group ($p = 0.001$), respectively.

5.3. NT-PRO-BNP AND CELL-FREE DNA IN ARF PATIENTS

Higher than normal (>125 pg/ml) NT-pro-BNP values were detected in 88 % (529 of 602) of study patients at baseline. Baseline levels were significantly higher in 90-day non-survivors than survivors, median (IQR) 4378 pg/ml (1400 – 13943 pg/ml) versus 1052 pg/ml (232 – 4076 pg/ml) ($p < 0.001$), and the difference remained significant for sample B on day 2, 3458 pg/ml (865 – 11875 pg/ml) versus 1613 pg/ml (359 – 3891 pg/ml) ($p < 0.001$), respectively.

NT-pro-BNP levels at baseline according to baseline levels of arterial oxygen pressure are presented in Figure 3. The difference between groups was significant, $p < 0.001$ (Kruskal-Wallis for all groups). Baseline NT-pro-BNP values in patients with normal renal function were significantly higher with chronic cardiac disease or cardiac surgery when compared to non-cardiac patients ($p < 0.001$). The levels of NT-pro-BNP increased significantly with deteriorating renal function in all patients ($p < 0.001$ for both cardiac and non-cardiac patients).

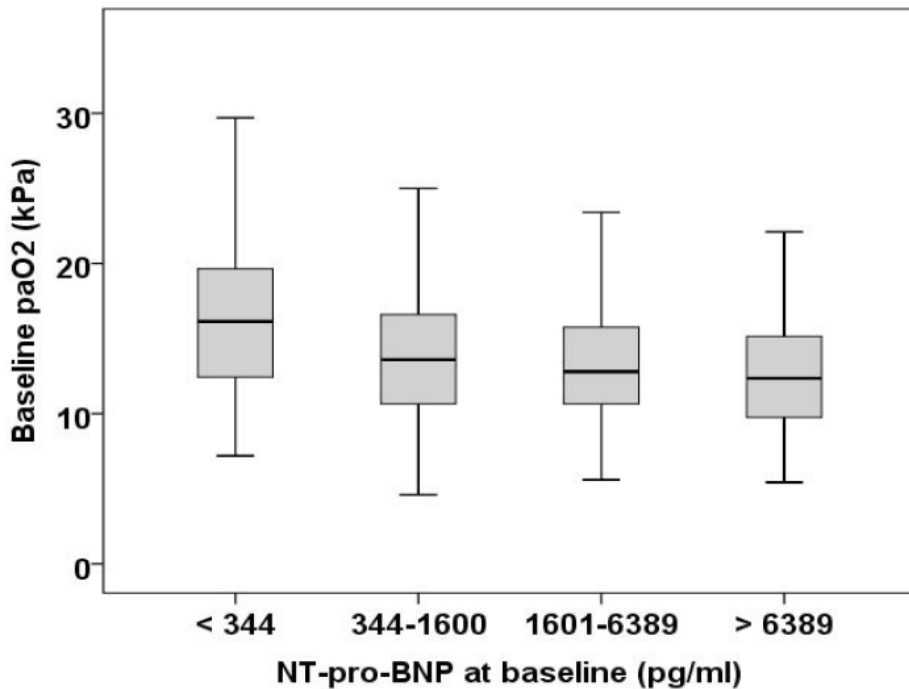


Figure 3. Baseline levels of arterial oxygen pressure (paO2) according to quartiles of baseline plasma N-terminal pro-brain natriuretic peptide (NT-pro-BNP).

Plasma cell-free DNA levels were significantly higher in 90-day non-survivors than survivors, median 16 636 GE/ml (IQR 7262 – 46 866 GE/ml) versus 10 026 GE/ml (4870 – 19 820 GE/ml), respectively ($p < 0.001$). The median (IQR) baseline DNA concentration in postoperative patients was 11 436 GE/ml (5587 – 20 379 GE/ml), and in non-operative patients 12 156 GE/ml (4938 – 27 292 GE/ml), $p = 0.36$. Plasma DNA at admission correlated with the baseline $\text{PaO}_2/\text{FiO}_2$ -ratio, $p < 0.001$. Figure 4 presents baseline plasma DNA according to quartiles of $\text{PaO}_2/\text{FiO}_2$ -ratio ($p < 0.001$, Kruskal-Wallis for groups).

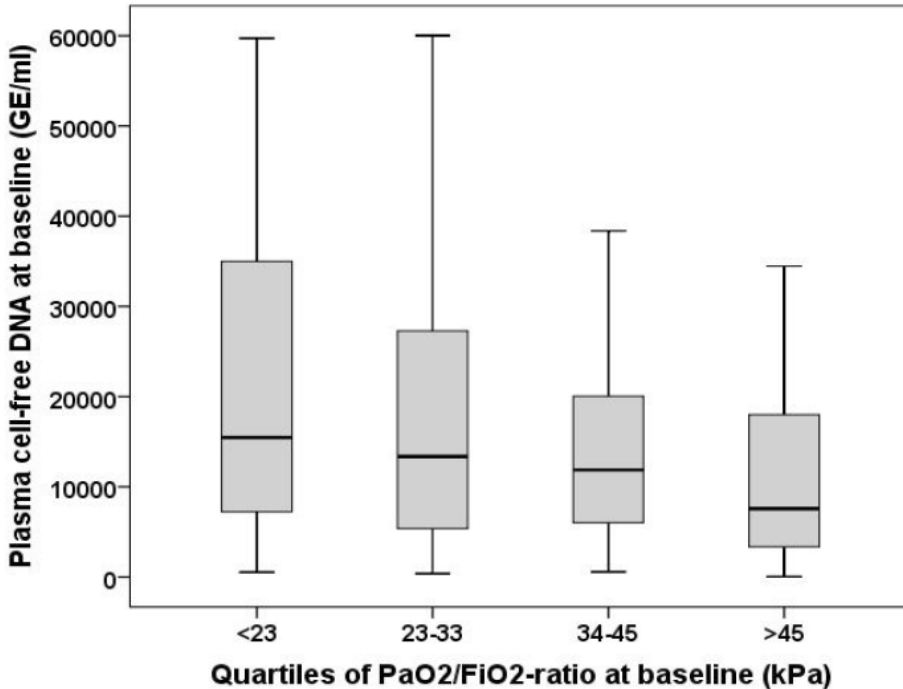


Figure 4. Plasma baseline cell-free DNA according to quartiles of $\text{PaO}_2/\text{FiO}_2$ -ratio

The ROC-curves of baseline NT-pro-BNP and plasma cell-free DNA for the 90-day mortality prediction are presented in Figure 5. The best cutoff value for NT-pro-BNP at baseline was 1765 pg/ml. Its sensitivity for 90-day mortality was 73% (95% CI, 66 – 79%), with a specificity of 63% (95% CI, 58 – 67%), a negative predictive value of 85% (95% CI, 81 – 89%), a positive predictive value of 44% (95% CI, 39 – 50%), and a positive likelihood ratio of 1.965 (95% CI, 1.686 – 2.289). The best cut-off value for baseline plasma cell-free DNA obtained from the ROC-curve analysis, was 16 000 GE/ml, with a sensitivity of 53% (95% CI 45 – 60%), a specificity 69% (65 – 74%), and a positive likelihood ratio of 1.72 (1.4 – 2.11). The odds ratio of plasma cell-free DNA being over cut-off value was 2.22 (1.41 – 3.48) for 90-day

mortality. The AUC of NT-pro-BNP levels at baseline was 0.718 (95% CI 0.674 – 0.761) and that of plasma cell-free DNA 0.624 (95% CI 0.572 – 0.676).

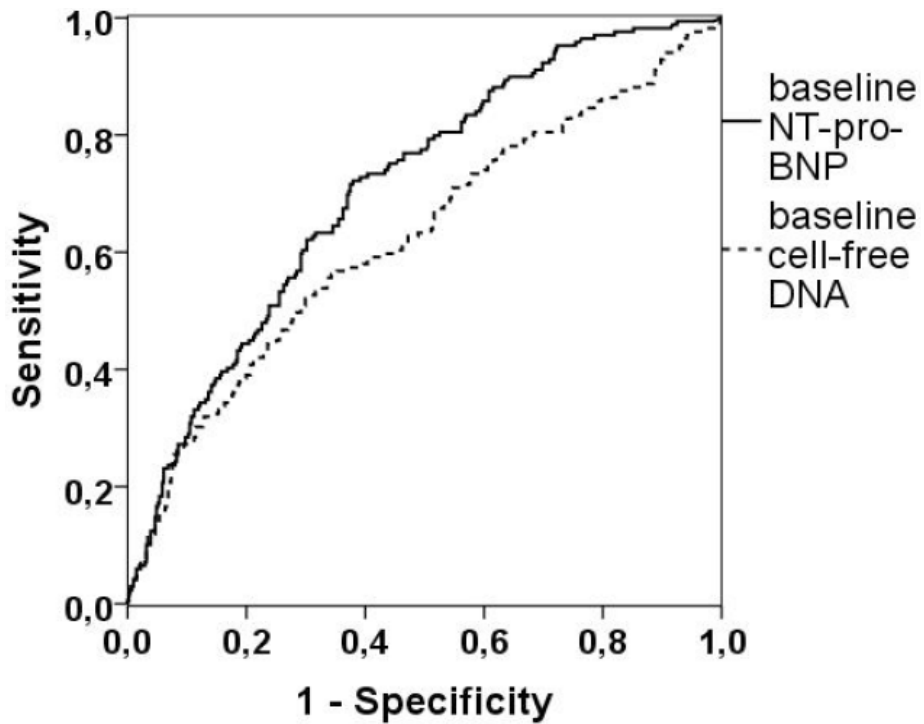


Figure 5. ROC curves of plasma baseline N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and cell-free DNA for predicting 90-day mortality

5.4. PROGNOSTIC VALUE OF COMBINING PLASMA NT-PRO-BNP AND CELL-FREE DNA

A significant correlation between NT-pro-BNP and cell-free DNA values was noticed at baseline, ρ 0.301, $p < 0.001$ (Figure 6).

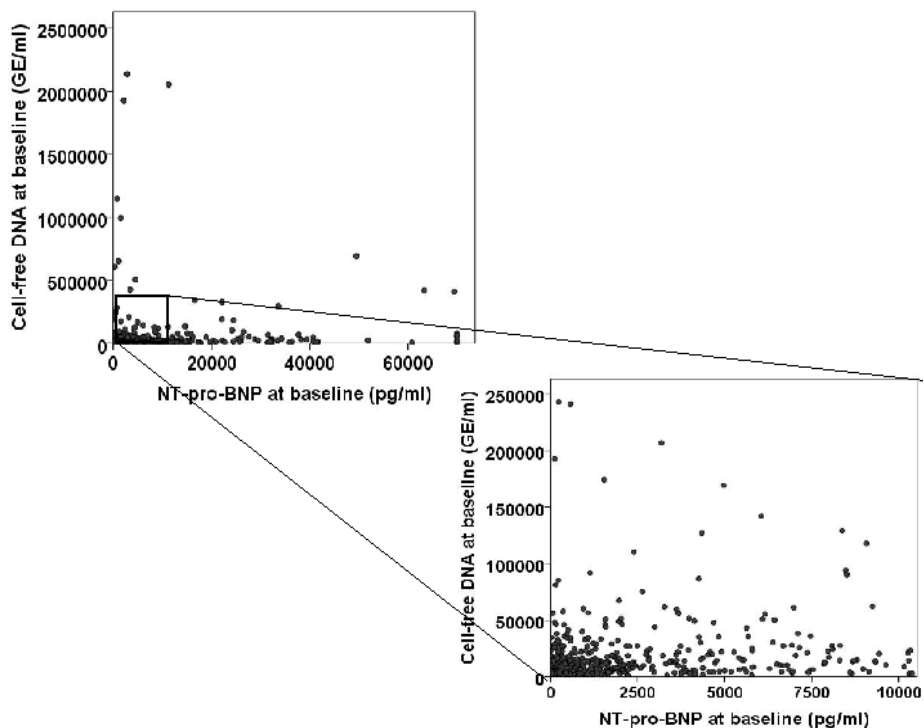


Figure 6. Plasma N-terminal pro-brain natriuretic peptide (NT-pro-BNP) values at baseline plotted against plasma cell-free DNA values at baseline. The picture in the upper left corner presents all values, and the picture in the low right corner a subset of values cropped from the upper picture.

The Kaplan-Meier survival curves for 90-day mortality in patients with baseline NT-pro-BNP and plasma DNA values over or below separate cut-off levels are presented in Figure 7. Survival was significantly different in patients with both markers being over cut-off levels ($n=132$), only NT-pro-BNP ($n=146$) or plasma DNA ($n=83$) being over cut-off, or both markers ($n=219$) being below cut-off levels ($p < 0.001$ log rank test for all curves).

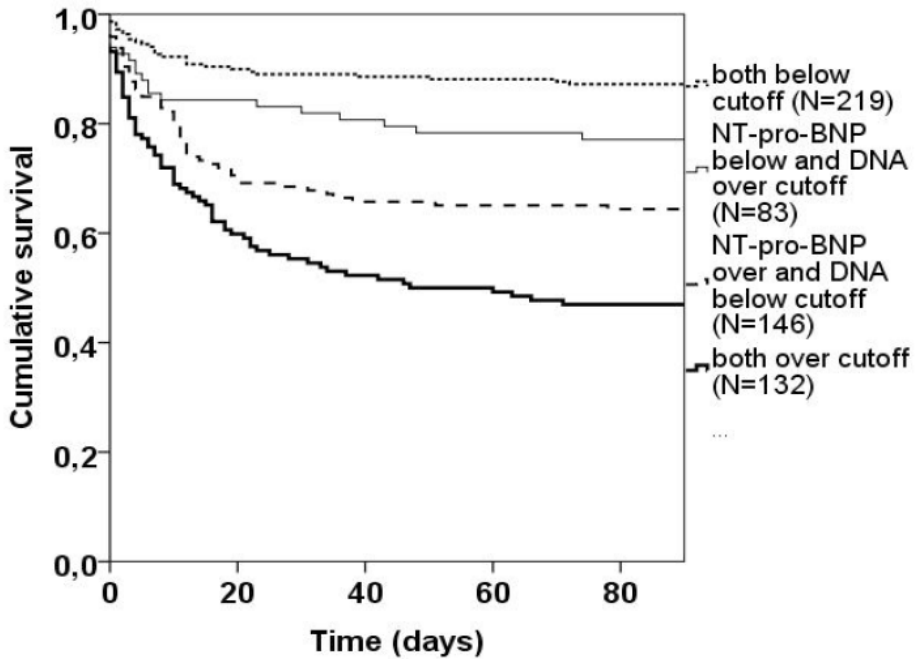


Figure 7. Kaplan-Meier survival curves for 90-day mortality according to both N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and cell-free DNA values being over or under cut-off

A multiple logistic regression analysis was accomplished with the inclusion of the following factors: baseline NT-pro-BNP over best cut-off (1795 ng/ml), baseline plasma cell-free DNA over best cut-off (16 000 GE/ml), infection status, baseline PaO₂/FiO₂-ratio, chronic heart disease, underlying risk factors for ARF (acute heart insufficiency, intoxication, and suspected aspiration), SAPS II score minus oxygenation, and SOFA score at 24 hours. The independent predictors of 90-day mortality were baseline NT-pro-BNP over cut-off ($p < 0.001$), baseline plasma cell-free DNA over cut-off ($p = 0.004$), baseline PaO₂/FiO₂-ratio ($p = 0.023$), and SAPS II score minus oxygenation ($p < 0.001$).

5.5. SERUM MARKERS OF COLLAGEN METABOLISM

Study patients ($n=68$) were on average 60 years old (SD 15, range 20-86), 50 (74%) were male, and 18 (26%) were postoperative patients. Thirteen (19%) patients fulfilled the criteria for ALI or ARDS, and 43 (63%) for MOD. SAPS II points ranged from 2 to 83 (mean 45, SD 15) and SOFA score at 24 hours from 2 to 17 (mean 9, SD 3). The mean (SD, range) ventilatory support time was 12 (11, 1-46) days, ICU

length of stay 15 (13, 1-60) days, and hospital length of stay 44 (20, 18-116) days. Patients in the cohort were more often admitted for operative reasons than the rest of the FINNALI study patients ($p = 0.026$). The ventilatory support time ($p < 0.001$), and length of stay in ICU and hospitals were significantly longer ($p < 0.001$ for both), but the severity of illness was equal, as measured by SAPS II score, and SOFA at 24 hours ($p = 0.36$ and $p = 0.43$, respectively).

PIIINP values increased over time in all patients in the cohort ($p < 0.001$), and the increase was more pronounced in patients with MOD ($p = 0.026$). The change in PINP levels over time was significant ($p < 0.001$), but did not differ between patients with MOD or without it ($p = 0.74$). ICTP levels showed a significant increase in all patients, but the levels in patients with MOD and without it were comparable ($p = 0.16$). In the maximum values of PIIINP, PINP, and ICTP during the first week, there was no difference between MOD and non-MOD patients.

Table 7 presents the levels of NT-pro-BNP and cell-free DNA in the collagen cohort patients in comparison to others. No differences in the levels were noticed between these groups.

Table 7. Plasma N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and cell-free DNA values in patients with both markers measured; a comparison between patients in collagen cohort and other patients. Values are presented in median (IQR).

		Patients in the collagen cohort	Other patients with NT-pro-BNP and cell-free DNA samples	p-value (Mann-Whitney)
Number		56	524	
NT-pro-BNP	at baseline	1646 (296 - 6438)	1626 (348 - 6423)	0.91
	at day 2	1874 (283 - 8251)	1882 (539 - 4869)	0.68
Plasma cell-free DNA	at baseline	14 540 (6490 - 27 556)	11 578 (5199 - 24 198)	0.20
	at day 2	13 370 (6982 - 25 623)	11 540 (6348 - 20 308)	0.26

5.6. OUTCOME

The overall mortality at 90-days was 31% (95% CI 28-34%). In a subgroup of patients with ventilatory support more than 24 hours, 90-day mortality was 32% (95% CI 29-36%) and in ALI/ARDS patients 47% (95% CI 35-59%). In patients with a failure of non-invasive ventilatory support, the mortality rate was 49%. Patient mortality at 30 days in study IV was 4% (3 of 68).

6 DISCUSSION

Incidence

The incidence of acute respiratory failure reported here is much higher than reported earlier (Lewandowski et al. 1995, Luhr et al. 1999). The most obvious reason for this is the wider definition used in this study. Although one earlier study has reported an ARF incidence of 137 / 100 000, which is closer to that of the present study, the definition used in that study was completely different (Bersten et al. 2002). Nonetheless, the incidence in the present study remained high, even after correction of the ARF definition to 24 hours of respiratory support. Other plausible explanations include differences in admission policies and organizational factors. In many European countries, specific respiratory ICUs and intermediate care units exist (Corrado et al. 2002, Polverino et al. 2010). In Finland, however, intermediate care units are uncommon, and, thus, patients needing ventilatory support are commonly admitted to ICU. Additionally, the differences in health care systems may partly explain these results, especially in comparison to north American studies. The incidence of ALI/ARDS observed, on the contrary, was lower than in some other studies (Bersten et al. 2002, Rubenfeld et al. 2005) but still at the same level as in an earlier report from Finland (Valta et al. 1999). The difference in ALI and ARDS incidence remains unexplained, but factors mentioned above, most probably have some impact. Genetic variation for susceptibility may also partly explain this variation (Marshall et al. 2002).

Ventilatory modes and settings

The proportions of ventilatory modes used at baseline, resemble those reported in a 1-day point prevalence study in Nordic countries (Karason et al. 2002). In the present study, the use of pressure-controlled modes was clearly more popular than in most countries outside Scandinavia (Esteban et al. 2008).

In Finnish ICUs, the lung protective ventilatory strategy has been adopted only partially, the result being uniform with earlier reports (Weinert et al. 2003, Young et al. 2004). A steady increase in a proportion of tidal volumes less than 6.5 ml/kg PBW has been reported previously, but the proportion was still around 40% of patients in 2005 (Checkley et al. 2008). The tidal volumes in the present study were

higher than recommended, especially when calculated per predicted body weight instead of actual body weight. The difference in tidal volumes between PBW and ABW was larger in women than in men, as reported previously (Gajic et al. 2004), which has an important clinical significance. The size of the lungs is determined by gender and height and these factors should primarily impact the tidal volume setting, a fact that is often forgotten in clinical practise. The limitation of inspiratory pressure was, however, much better implemented.

NT-pro-BNP and cell-free DNA in ARF patients

Both of the evaluated biomarkers were commonly increased in critically ill patients with acute respiratory failure. The levels of NT-pro-BNP were elevated in the great majority of the ARF patients, which is in accordance with earlier studies reporting great increases in NT-pro-BNP levels in ARF and ARDS patients (Bajwa et al. 2008). The present study differs from earlier studies in the patient population, but the results are basically comparable. Reference values for plasma cell-free DNA do not exist, but the levels in this study were high when compared to normal controls in meta-analysis (van der Vaart et al. 2010). When comparing to earlier studies, our results are essentially in accordance. Because of variations in method, however, direct comparisons cannot be done.

Cut-off values of NT-pro-BNP for best mortality prediction have ranged from 940 pg/ml in an unselected, non-cardiac ICU patient population, to 6800 pg/ml in ARDS patients, and 7090 pg/ml in patients with severe sepsis and septic shock (Varpula et al. 2007, Bajwa et al. 2008). The best cut-off value in the present study was 1765 pg/ml, in comparison to a previous study of 78 ICU patients (with an APACHE II score over 12) that had a level of 1900 pg/ml (Almog et al. 2006). The AUC for baseline NT-pro-BNP for the prediction of 90-day mortality in this study was 0.718. This result is in accordance with earlier studies in ARDS (Bajwa et al. 2008, Kotanidou et al. 2009).

The exact mechanism by which NT-pro-BNP is elevated in ARF patients is not known. Myocardial function, and thus the release of NT-pro-BNP, may be affected by acute respiratory failure and subsequent ventilatory treatment in several mechanisms. Theoretically, increased NT-pro-BNP could serve as a marker of fluid overload. Earlier studies, however, have shown that NT-pro-BNP levels are not able to distinguish between cardiogenic pulmonary oedema and ALI (Levitt et al. 2008) and do not associate with pulmonary artery occlusion pressure in critically ill patients (Jefic et al. 2005, Tung et al. 2004). The results of the present study are in accordance, since no correlation between baseline NT-pro-BNP and central venous pressure (CVP) or pulmonary artery occlusion pressure (PAOP) was noticed.

Furthermore, filling pressures do not necessarily correlate with cardiac preload in critically ill patients, a result reported in mechanically ventilated sepsis patients (Osman et al. 2007). The relationship between NT-pro-BNP and the elevation of intrathoracic and transpulmonary pressures induced by positive pressure ventilation remains unresolved.

NT-pro-BNP levels have been reported to increase in patients with renal insufficiency (Forfia et al. 2005b) and the results of this study are compatible. NT-pro-BNP values showed a clear tendency to increase with deteriorating renal function whether the patient had a cardiac condition or not. In many critically ill patients, the significance of NT-pro-BNP measurements is, thus, diminished.

An interesting issue is the possible role of myocardial hypoxia. Hypoxemia has been shown to stimulate the secretion of BNP in an animal study on piglets (Khan et al. 2008). Additionally, hypoxia has been shown to directly induce BNP release from human myocytes in patients with cyanotic congenital heart disease (Hopkins et al. 2004). BNP has also recently been shown to be able to detect silent myocardial ischemia in diabetic patients without heart insufficiency (Rana et al. 2006). Based on recent data, the role of hypoxia has been emphasized in the regulation of natriuretic peptides (Arjamaa et al. 2011). The results of the present study are in accordance with this hypothesis, since the baseline NT-pro-BNP levels increased with the lowering arterial oxygen pressure, although the median levels were not hypoxemic. The level of tissue hypoxia, however, was not measured in this study. In critically ill patients, especially in septic patients, the oxygen utilizing capacity of tissues, resulting from the deteriorated microcirculation, is known to decrease, resulting in lactatemia and risk of organ dysfunction (Levy et al. 2005).

BNP release has also been shown to be stimulated by proinflammatory cytokines (Ma et al. 2004) and several neurohormones (adrenergic agonists, endothelin, and ATII) (Mair 2008). Theoretically, these factors may have played a role in our study population and partly explain the increased concentrations of NT-pro-BNP.

A range of different methods used in the quantification of cell-free DNA in earlier reports makes it difficult to make direct comparisons between studies. These differences most probably play role in some studies with highly differing concentrations (Kocsis et al. 2009). The levels of plasma cell-free DNA in this study, in general, are situated in the middle of those reported previously (Rhodes et al. 2006, Saukkonen et al. 2008, Saukkonen et al. 2007). In non-operative patients, baseline plasma DNA levels were higher in those with infection, consistent with published results (Rhodes et al. 2006, Saukkonen et al. 2007). In operative patients, the levels did not differ, although there was a tendency ($p = 0.053$) towards it. Operative patients presented with less severe illness, as measured by SAPS II score, but had similar levels of plasma DNA in comparison to non-operative patients. This result suggests that surgery, per se, may have raised the levels. This hypothesis is

supported by the rising plasma DNA in operative, but not non-operative patients.

The prognostic value of plasma DNA was not particularly good in this study. The AUC was 0.643 for the 90-day mortality prediction in all patients in the cohort, somewhat lower than previous studies. None of the subgroups presented with better values. When examining the earlier reports of plasma DNA in critically ill patients, it is obvious that AUC is better when tested on short period survival, but shows a tendency to lower when longer outcome periods are employed (Rhodes et al. 2006, Saukkonen et al. 2008). In this context, the result of the present study is consistent.

The exact origin of plasma cell-free DNA in this study remains unclear. Theoretically, possible options in ARF include apoptotic neutrophils, alveolar epithelial cells, and myofibroblasts, but also remote organ cells. Furthermore, unlike in healthy humans, cell necrosis associated with inflammation is probable. A first successful attempt to characterize circulating DNA in cancer and control patients was made recently, where a differentiation between groups could be shown (van der Vaart et al. 2008). Tissue hypoxia is one tempting reason for increased plasma DNA levels, supported by a recent report in patients with mesenterial ischemia (Arnalich et al. 2010a). The higher concentrations in patients with infection support this hypothesis, considering the metabolic consequences of sepsis at the tissue level, as indicated earlier with NT-pro-BNP. Unfortunately, however, the tissue hypoxia parameter, lactate, was not measured in this study. Correlation with plasma DNA and pH at baseline was noticed, a finding possibly reflecting anaerobic metabolism at the tissue level. Nonetheless, due to observational nature of this study, these speculations remain purely hypothetical.

Prognostic value of combining plasma NT-pro-BNP and cell-free DNA

A correlation between baseline levels of NT-pro-BNP and plasma cell-free DNA was found, but it was rather weak, revealed also by visual inspection (Figure 6). Contrary to this, in a recent small study, no correlation between plasma DNA and NT-pro-BNP was noticed (Rhodes et al. 2006).

In a logistic regression analysis including both markers as categorical variables, they remained independent predictors of outcome, suggesting a role separate from the severity of illness. $\text{PaO}_2/\text{FiO}_2$ -ratio was also an independent predictor in the multivariate analysis of study I without any biomarkers included in the analysis. Earlier studies have shown differing results concerning $\text{PaO}_2/\text{FiO}_2$ -ratio as a prognostic factor in ALI and ARDS patients. Measured at baseline, it has not been an independent predictor of death, although it may have been associated with death in univariate analysis (Brun-Buisson et al. 2004). The other factors remaining significant in multivariate analysis were SAPS II score minus oxygenation points,

SOFA score at 24 hours, infection status, and chronic heart disease, the result being in accordance with earlier studies. SAPS II, in particular, has been frequently found to independently predict mortality in ALI/ARDS patients (Brun-Buisson et al. 2004, Monchi et al. 1998, Nuckton et al. 2002). In addition, chronic diseases, sepsis, and organ dysfunctions have been reported to be prognostic factors (Brun-Buisson et al. 2004, Doyle et al. 1995, Zilberberg et al. 1998).

A significant difference in survival was noticed, when both biomarkers were used in combination (Figure 7), consistent with a recent study evaluating the use of several biomarkers in combination for the diagnosis of ALI in trauma patients (Fremont et al. 2010). In that retrospective analysis, 107 trauma patients fulfilling AECC criteria for ALI/ARDS during their first three days after admission were compared to 85 patients without ALI over seven days. From 21 biomarkers measured, 7 markers representing epithelial and endothelial injury, collagen deposition, cardiac dysfunction, and inflammation, were selected to form a biomarker panel. Using this panel for diagnosis of ALI/ARDS, the AUC was 0.86 (95% CI 0.82-0.92). Restricting the number of biomarkers in the panel to three best performing (RAGE, procollagen propeptide III and BNP), the AUC was almost at the same level, 0.83 with 95% CI 0.76-0.88. Patients with a high probability of ALI/ARDS, according to the panel, spent more days in the ventilator and in ICU when compared to patients with a low probability of ALI/ARDS. The hospital mortality did not differ between groups, 18% in high and 6% in low probability group, but the study was most probably underpowered for the prediction of mortality. Another recent study showed similar results for predicting the mortality of ALI/ARDS patients with several biomarkers (Ware et al. 2010). This study was based on a patient cohort of an earlier prospective study of 549 patients, where low versus high levels of PEEP were evaluated (Brower et al. 2004). Ware and colleagues observed that a combination of six clinical risk factors and eight biomarkers was superior in mortality prediction with AUC 0.85 (95% CI 0.813 – 0.833). A reduced model with two clinical risk factors (APACHE III score and age) and two biomarkers (SP-D and IL-8), showed almost as good predictive power with AUC 0.834 (95% CI 0.789 – 0.862).

Markers of collagen metabolism

This was the first study to report the levels and the evolution of markers of collagen metabolism in patients with ARF. Earlier studies in ARDS patients have found procollagen III levels to increase early in the course of disease and the results of this study were comparable (Marshall et al. 2000, Meduri et al. 1998). In ARDS patients, increase in PINP and PIIINP over time has been reported, in accordance with the results of the present study (Meduri et al. 1998). PINP levels, although

tending to increase, remained practically inside reference values. A similar result has been reported in severe sepsis patients (Gaddnas et al. 2009). The ratio of PIIINP and PINP increased early, followed by a later decrease back to baseline. This suggests that the collagen profile changes to a more stable and less deformable composition over time, a novel finding in this study. ICTP levels also showed an increase over time, that when accompanied with an initial decrease in the ratio of PINP and ICTP, this suggests degradation of collagen type I during the acute phase of ARF. High ICTP levels have been previously reported in patients with trauma and severe sepsis (Gaddnas et al. 2009, Waydhas et al. 1993).

Whether patients suffered from multiple organ dysfunction or not, maximum levels of serum markers of collagen metabolism did not differ. This is inconsistent with a previous report in sepsis patients (Gaddnas et al. 2009). The highly selected patient population in the present study is most probably a reason for this discrepancy. Only patients with serial blood samples extending to day 21 were included. Inevitably, this excluded the most severely ill patients, who did not survive until day 21. For the same reason, the mortality of study patients was extremely low, and precludes prognostic analyses.

Due to small patient numbers, subgroup analyses concerning operative status and the use of corticosteroids were not rational. These factors, however, may have an effect on procollagen levels. Procollagens measured from plasma have been shown to increase early in lung injury, correlating with outcome (Meduri et al. 1998). In this small landmark study of Meduri and colleagues, treatment with methylprednisolone resulted in the decrease of procollagen levels and increased survival. Furthermore, in a small clinical trial where corticosteroid treatment was started 10 days after the primary insult in ALI patients, better oxygenation was seen in the steroid group, but no difference in mortality nor adverse effects (Varpula et al. 2000). More recently, the results of a large scale RCT with 180 patients have been reported (Steinberg et al. 2006). In that study, patients in the methylprednisolone group had improved oxygenation and lung compliance, and fewer days in ventilator, but also higher rate of recurrent respiratory failure and no difference in mortality. Corticosteroid treatment was started, however, not until one to four weeks after the diagnosis of ARDS, possibly too late in the light of studies showing collagen synthesis to start very early after primary insult (Marshall et al. 2000). Of special interest, in a post-hoc analysis, patients treated with steroids and having higher levels of procollagen III at baseline, experienced better survival, in contrast with patients with low procollagen III levels, in whom steroid treatment associated with higher mortality. This result supports the wider employment of individually customised treatment for which biomarkers are one tool that may guide clinical decisions.

Outcome

The overall mortality at 90-days was 31%, which is slightly lower than in earlier studies in ARF patients (Behrendt 2000, Luhr et al. 1999), thus the implementation of lung protective ventilatory strategy may impact clinical outcome. On the other hand, mortality in ALI and ARDS patients was higher than in some randomized trials but comparable to recent pooled mortality in observational studies (Phua et al. 2009). It is worth noting that in multicentre studies, only Luhr and colleagues have reported 90-day mortality previously, the same end point that has been utilised in the present study.

Strengths and limitation of the study

The unquestionable strength of this study is a large and nationally comprehensive patient material. All intensive care units, except two units from small central hospitals, participated in this study. The coverage of 25 participating units represents 97% of the adult population in Finland. Thus, the generalisation of the results can be considered quite good in the group of critically ill ARF patients. However, the study has several points that need to be discussed. First, the group of ARF patients is heterogeneous due to numerous diseases able to cause respiratory failure. ARF does not have any standard definition, and thus the definition used in the present study is also arbitrary and can be criticised. All patients needing respiratory support, invasive or non-invasive, for at least six hours were included. The time frame has been longer in earlier studies, 24 hours in most cases, and only patients with invasive ventilation had been included (Flaatten et al. 2003, Luhr et al. 1999). However, in order to evaluate prognostic laboratory markers (Studies II and III), it is logical to collect the samples as near the insult as possible. The exact time of an insult leading to acute respiratory failure, is in most cases impossible to determine. The time elapsing from insult to sampling is, thus, imprecise and variable. Ventilatory support itself can also be considered an insult, therefore, minimizing the time of ventilatory support is rational in this context. Furthermore, excluding non-invasively treated ARF patients from the study would not have been sensible, since they clearly form a subgroup of ARF patients. Non-invasive ventilatory support has been used increasingly during previous years (Demoule et al. 2006) and has proven to be superior to invasive ventilation in selected groups of patients (Hilbert et al. 2001, Plant et al. 2000). Additionally, the results of the subgroup analyses with stricter ARF criteria, closer to the criteria of earlier studies, did not differ from the results of all patients strengthening the generalisation of the results.

The patients included in studies II and III, and especially in study IV, represented only a subgroup of all patients. Patients were more often admitted for elective

surgery in studies II and III, which may impact the results. In subgroup analyses performed in studies II and III, the results did not change, although elective patients and patients with a ventilatory period of less than 24 hours were excluded. Patients in study IV were stringently selected in order to evaluate the development of fibrosis markers over time, which prevented reasonable analyses concerning outcome.

Ethical considerations

The study was approved by the ethical committee of Helsinki University Central Hospital. Due to the observational nature of the study, based on an established standard of care, informed consent was required only for blood sampling. Blood sampling was accomplished via arterial line, which is an established part of the monitoring in intensive care units. All patients in this study were treated with respiratory support, most with invasive ventilation, and sedation that rendered them incapable of giving consent. Thus, after explaining study information verbally and in writing, a written consent was obtained from the next of kin. Given that the next of kin may be suffering some confusion during the acute situation, this approach could be seen as ethically vulnerable, but the negligible risk caused by the blood sampling attenuates it.

Methodological considerations

This was a prospective cohort study, which is reasonable for evaluating the questions posed in this study. Some issues, however, need to be discussed. This study was prospective, but purely observational, and patient care was not standardised in anyway. Factors known to have effect on outcome have been taken into consideration in the statistical analyses, but many unknown factors may have had an impact on the results. Additionally, the observational nature of the study makes it impossible to confirm or refute any causalities. The challenge of a cohort study is to collect a patient population which would represent well the larger population. The size of the cohort should be large enough, both in number of patients, and in geographical coverage. In this study, the overall number of patients was sufficient, but numbers in subgroups, including ALI and ARDS, was considerably lower restricting the confirmative subgroup analyses. The geographical coverage was excellent and possible impact factors, such as associated genetic factors among others, can be considered minimal.

The duration of the study period was eight weeks and the incidence calculations were based on that. Periodical change in the incidence is, thus, possible and can

be reflected to results. The occurrence of respiratory infections, in particular, is an important underlying condition for ARF, and is thought to show some seasonal variation. Indeed, the number of patients admitted to Finnish ICUs due to respiratory failure has been shown to be higher in the winter time (Reinikainen et al. 2006). The patient cohort in the present study was collected in the late spring time, so the possible bias would cause some underestimation of the incidence.

The laboratory method used in the analysis of NT-pro-BNP was a routinely-used commercial method. All samples were collected to Helsinki and the analyses were performed in one laboratory (Helsinki University Central Hospital Laboratory, HUSLAB) by professional personnel. Standardised laboratory methods for plasma cell-free DNA extraction and determination were carried out by experienced laboratory personnel. The measurements of serum markers of collagen metabolism were accomplished by a specialised laboratory in Oulu University Hospital.

Clinical implications and future perspectives

The results of this study are important in many aspects. First, the results of the incidence of ARF (I) offer valuable information regarding organizational planning, especially concerning the need for intensive care beds. Second, the ability of NT-pro-BNP to differentiate between cardiac and non-cardiac reasons for respiratory failure is affected by renal failure, suggesting that this measurement has limited value in many critically ill patients. Third, either NT-pro-BNP or plasma cell-free DNA as single parameters, do not seem to work as prognostic tools in population of unselected ARF patients, but when used in combination, the value seems to be better. Finally, interesting results of increased collagen III synthesis in ARF are worth further studies.

Since ALI is a multifactorial syndrome, with differences known to exist in the pathophysiology, and in the clinical picture according to predisposing conditions, it would seem rational to account for these differences somehow in future studies. Measurements of lung mechanics would be one solution to differentiate patients with different forms of ALI (Gattinoni et al. 1998). Standardized performance of these procedures may be challenging in a clinical setting, although new technology has been introduced recently for monitoring of ventilated lung areas (Frerichs et al. 2002) and functional residual capacity (FRC) (Wrigge et al. 1998). In large multicentre studies however, the use of this technology, with variation between manufacturers would be challenging.

Measuring biomarkers offers another possibility to recognize and stratify ALI patients better in future randomized trials. Large and heterogeneous patient populations provide more power for mortality prediction, but smaller, more

selected and homogeneous populations with more severe disease, and possibly greater treatment benefit, would be rational, especially in trials testing new therapies. Finding those patients who are most likely to benefit from potential therapy is essential. An example reflecting well on the significance of better stratification of patients in the studies is the finding that plasma RAGE associates with mortality only in patients with injurious tidal volumes (Calfée et al. 2008). Thus, the value of RAGE as a prognostic measure, seems poor in the present standard of care (lung protective ventilatory strategy). Of single biomarkers tested in large trials, none has proved to be excellent, and a combination of several biomarkers representing distinct aspects of the pathophysiology of ALI has been reported to be superior in mortality prediction (Ware et al. 2010). In addition to being potential tool in research, measurements of biomarkers could still provide a clinical tool for monitoring the course of disease or response to treatment.

An important future direction is to refine the criteria for ALI and ARDS. The AECC criteria, which have been established for almost 20 years, have not been able to achieve what they were initially intended for. As noticed already more than a decade ago, the use of these criteria in two concurrent studies resulted in two different kinds of ALI/ARDS patient populations, thus divergent results (Roupie et al. 1999, Villar et al. 1999). Additionally, the occurrence of ARDS has shown wide variation between centres even inside a single study, although the same criteria had been used (Brun-Buisson et al. 2004). Indeed, new criteria for ARDS have just been presented at the annual meeting of the European Society of Intensive Care Medicine (ESICM 2011, Berlin). ARF patients have a significant impact on healthcare, thus if the standard criteria for this condition could be defined, the research field would encompass a larger defined patient population.

A well recognized existence of differences in the clinical picture of ALI, as well as in the susceptibility to develop ALI when exposed to risk factors, suggests a role for genetic variants. Studies of genetic polymorphisms have revealed several points in ALI pathogenesis, including ACE, IL-10, and TNF (Gong et al. 2006, Gong et al. 2005, Marshall et al. 2002). Also, polymorphisms associated with genes regulating the coagulation cascade are potentially important in ALI, as has been characterized well earlier (Aiach et al. 1999, Arnaud et al. 2000, Endler et al. 2003). Studying the genetics of such a complex disease as ALI is challenging, in fact the genetic research of ALI remains in its infancy (Gao et al. 2009). A paucity of replication studies, which constitute to confirm the genotype-phenotype associations, is a common problem in the growing field of clinical genetic research in general and also with studies concerning ALI (Chanock et al. 2007). Genetic research of ALI is essential to fully understand the big picture of this syndrome, and will definitely be increasingly pursued in the future.

Although much remains to be accomplished in future work, the studies of biomarkers has increased the knowledge of ALI pathophysiology enormously. A big dilemma remains, that smaller experimental studies, which can better measure the consequences of interventions and physiological incidents, often lack the power to show more than associations between matters. Furthermore, huge resources are needed to perform larger studies of reasonable study periods, with enough power for prognostic purposes, but also a patient population that is selected in a rational way.

7 CONCLUSIONS

Following conclusions can be drawn on the basis of these studies:

1. The calculated incidence of acute respiratory failure in Finnish ICUs was 149.5 / 100 000 inhabitants per year. The 90-day mortality was 31%. Tidal volumes were larger than recommended. In inspiratory pressures the recommendations were implemented better.
2. Plasma NT-pro-BNP and cell-free DNA measured at baseline were commonly increased in ARF patients. Renal function and cardiac condition had impact on NT-pro-BNP values. Both NT-pro-BNP and plasma cell-free DNA at baseline were independent predictors for 90-day mortality. NT-pro-BNP and plasma DNA correlated with each other at baseline, and their use in combination may increase their prognostic value.
3. The first week of ARF was represented by increased synthesis of collagen type III and degradation of collagen type I. Three weeks after ARF commenced, collagen metabolism reverted towards normal. No association with multiple organ dysfunction was noticed.

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